FINAL REPORT

East Texas Threatened Mussels
Project Period: June 2016 to December 2017
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Introduction

This work presents two research tasks that were an add-on to our previous Texas Comptroller grant research for East Texas Threatened mussels (Ford et al. 2016). These new tasks allowed us to answer more specific questions that were pertinent for listing consideration or potential designation of critical habitat. We addressed specific questions about mussel response to environmental stressors for the candidate species in East Texas Rivers. This new phase of research began in July 2016 and continued to December 2017.

Task 1. Physiological responses of *Fusconaia* to environmental stressors

East Texas Rivers are impacted by anthropogenic effects of pollution, temperature and flow alteration from dams and climate change. Populations of mussels in rivers are highly susceptible to environmental alterations because of their primarily sessile behaviors and filter-feeding ecology (Fritts et al. 2015). The individual effects of these factors can be lethal and reduce populations directly or by reducing numbers over time through sublethal effects on reproduction and long-term survival. Although some recent research on the effects of elevated temperature, dewatering, agriculture and oil field run-offs, pharmaceuticals and other pollutants in waste water, and other environmental stressors such as sediment load have been conducted on mussels (Goodchild et al. 2016, Gillis et al. 2014, Pinkney et al. 2014). The number of species tested is very limited relative to the high diversity of the family. In addition, effective methods to determine sublethal effects are both difficult and expensive. We evaluated three factors likely impacting mussels in east Texas, the effect of elevated temperature, nitrogen and siltation (a surrogate for bank erosion) and examined both lethal and sublethal levels of these stressors. We also used ecological niche modeling (Maxent software; Phillips and Dudik 2008) to assess landscape level effects of environmental stressors (e.g., number of oil rigs, distance from dam, agricultural run-off) on the occurrence of *Fusconia*, *Pleurobema,* and *Potamilus amphichaenus* in East Texas.

Methods

Experimental Design

Environmental factors likely having the greatest impact on East Texas mussels include increased temperatures, excess nitrogen exposure, and siltation (Burlakova et al. 2011, Randklev et al. 2016). The laboratory at the University of Texas at Tyler is equipped with two large aquatic flow-through tanks that provide optimal conditions for studying freshwater mussel behavior and response to changing and/or controlled conditions. These tanks can be adapted with a heating and cooling system, and are large enough to hold the amount of sand required to mimic field bank collapse conditions for studying siltation. Additionally, there is a large thermally regulated cooler which can be used to house a smaller, contained flow-through system for holding and acclimating mussels in the lab.

Mussel Collection and Housing

Adult mussels were collected during 2016 and 2017 from two high density sites on the Sabine river near Hawkins, Texas, known to have an abundant and reproducing population of *Fusconaia askewi* (Burlakova et al. 2012, Ford et al. 2009). Mussels were transported back to the lab at the University of Texas at Tyler within 1 hour of removal from stream in coolers wrapped in towels wet with river water. Upon arrival at UT Tyler all individuals were weighed, measured to the nearest mm, tagged and placed in a temperature controlled, flow-through acclimation chamber at 20 degrees Celsius for at least 2 weeks before any testing began. Mussels were fed 500 ml daily from a stock of Reed Mariculture (6ml of Nanno 3600 and 12 ml of shellfish diet 1800) (ASTM 2006, Ganser et al. 2015).

Task 1.1. Elevated Temperature Study

The effects of elevated temperature on *Fusconaia askewi* were tested through exposure of a total of 84 individuals (21 replicates per treatment) to water temperatures of 20, 25, 30, and 35 degrees Celsius over 21 days. Twenty degrees Celsius represented the baseline temperature, and mussels were acclimated up to the test temperature by increasing water temperature $\leq$ 3 degrees Celsius per day (Ganser et al. 2013). Every seven days, one third of the mussels per treatment were randomly selected for analysis. Tissues samples were collected to test glycogen as a stress indicator over time. Glycogen levels were determined by chemical extraction from tissues followed by colorimetric absorbance on a spectrophotometer (Naimo et al. 1998). Tissue samples were stored at -80 degrees F until analysis. Additionally, mortality was recorded daily and LC50's used to determine the lethal range of temperatures for adult mussels (Pandolfo et al. 2010). An iButton (iButtons, Alpha Mach, Inc. Mont St-Hilaire, QC, Canada) was submerged in the tank during the entire trial to record temperature readings every minute for the duration of each trial (Ganser et al. 2015). Daily water quality monitoring was conducted with a Hydro Tech HYDROLAB Compact DS5 for temperature, pH and dissolved oxygen. Conductivity, and salinity were monitored weekly following the Standard Guide for Conducting Laboratory Toxicology Tests with Freshwater Mussels (ASTM 2006). Temperature in the experimental flow-through tanks was controlled within 1 degree Celsius by using an Arctica Titanium Chiller and a Process Technologies 1800-watt, 120-volt industrial heater, both with digital temperature control.

Task 1.2. Siltation Study

The effect of siltation on *F. askewi* was evaluated as a surrogate for bank collapse in field conditions. Mussels were placed in a flow-through tank and completely buried with sand at depths of 0.25 and 0.5 Meters and then subsets excavated daily to monitor health by examining mortality and glycogen levels, which is a known stress response in Unionids (Naimo et al. 1998). Twenty individuals were buried at each depth, with an additional 20 on top of the sand as control. Fifteen individuals (5 from each treatment) were removed at 24, 48, 72, and 96 hours. All mussels had individual tags on their surface and a 1.5 Meter filament line attached to a corresponding floating tag on the end. This is how the mussels were identified while under the sand. Mussel movement was determined with a polyvinyl chloride (PVC) grid, used to note initial location and then overlaid daily to determine horizontal movement by measuring changes in filament line position (Allen and Vaughn 2009). Measurement of filament line above the sand was used to determine vertical movement. The water in the tank continuously flowed to simulate a natural environment.

Task 1.3. Nitrogen Toxicity Study

In determining the acute effects concentrations for invertebrates in the most recent report by the United States Environmental Protection Agency, data were normalized to a pH of 7 and a temperature of 20 degrees Celsius (U.S. EPA 2013). Temperature and pH change the proportion of toxic to non-toxic forms of ammonia, and were therefore monitored and controlled at the aforementioned parameters throughout the experiment to limit their effects. Other water chemistry parameters monitored daily were dissolved oxygen and specific conductance. Because the toxic effects of ammonia have been shown to be lessened in experiments conducted with sediment (Newton and Bartsch 2007), all toxicity trials were conducted in eight 20-gallon tanks with 2 inches of river rocks to approximate the bottom of the Sabine river where the mussels were collected. Twelve animals (3 replicates of 4 individuals per trial) were exposed to each of six concentrations of ammonia. Ammonium chloride is the source of nitrogen, and concentrations used were as follows: 0, 6.25, 12.5, 25, 50, and 100 mg/L total ammonia nitrogen (TAN) (Scheller et al. 1997). Mussel behavior was observed in each trial once every hour to determine amount of gaping, burrowing, moving (inactive, or relocated), and righting behaviors (realigning to a vertical position). Methods used by Bringolf et al. (2010) and Waller et al. (1999) were used and slightly modified in developing this observational format. Observations took place between 8 am and 5 pm for the duration of each 96-hour trial. One of the three trials in each concentration had a GoPro camera mounted in the tank recording behavior in two-hour segments. Between each two-hour segment, when the camera must be recharged, the researcher physically observed the tank until the camera could be replaced. This is in addition to the researcher observing mussels in all trials once every hour. Mortality was calculated as percent dead at 24, 48, 72, and 96 hours. Finally, tissue samples were taken for glycogen analysis.

Task 1.4. Ecological Niche Modeling

We used landscape environmental layers in a Geographic Information System (GIS) to create habitat suitability maps (also known as ecological niche models) for the six state-threatened mussel species in east Texas. This study focused on the associations of environmental factors with mussel distributions at a 100 m x 100 m resolution, where local habitat parameters including water velocity, depth, and substrate type are commonly thought to influence mussel abundance and distribution (Vannote and Minshall, 1982; Holland-Bartels, 1990; Strayer and Ralley, 1993; Strayer et al., 1994).

The ecological niche modeling software that we used, Maxent, produces a geographic model of habitat suitability by searching for the best solution that matches the distribution of the observed occurrences to the environmental variables (i.e., ArcGIS layers) (Phillips et al., 2006). It produces a map with a logistic score for each grid cell (corresponding to the resolution of the environmental data), which can be interpreted as the degree of suitability of a particular location for the species, given the environmental attributes of that location and their similarity to other locations where the species is known to occur (Phillips and Dudik, 2008). These habitat suitabilities, covering every place along the rivers at the resolution of our analysis, range from 0 to 1 with 0 representing the least suitable habitat and 1 representing the most suitable habitat. The analysis was restricted primarily to the Trinity, Cypress, Sulphur, Sabine, Neches, and Angelina rivers and their associated watersheds. Habitat suitability models were built separately for each species. To minimize autocorrelation at 1 km, we used the 'thin' function of the package spThin (Aiello-Lammens *et al.,* 2015) in R version 3.3.3 (R Development Core Team 2017) to remove all but one entry within that radius. Once the locations were thinned, there were a total of 82 locations for *F. askewii*, 35 locations for *P. riddellii*, and 39 locations for *P. amphichaenus*.

In previous models we used nine continuous environmental variables were incorporated into the model for each species: available water content in the surrounding soil (in/in), bulk density of the surrounding soil (in g/cm^3), the percentage of the surrounding soil consisting of clay, the percentage (by weight) of the surrounding soil consisting of organic matter, the erodibility factor (k) of the surrounding soil from the Universal Soil Loss Equation (USLE; Ontario Ministry of Agriculture, Food, and Rural Affairs, 2015), slope of the map unit as a percentage, the mean annual ground water recharge of the stream/river at that location (mm/year), the velocity of the stream/river at that location (ft/s), and the flow volume of the stream/river at that location (ft^3/s). We added 3 new layers for this project. These included sinuosity (USGS basin characteristics layer), agricultural chemical (% fertilizer, USGS), and oil and gas locations (USGS). Soil characteristics were obtained from the State Soil Geographic (STATSG0) Data Base (United States Department of Agriculture, 1994), and the data processing steps used to make this dataset are described in Wolock (1997). The hydrology layers were obtained from the NHDFlowline dataset (USEPA and USGS, 2005). These environmental variables were chosen because we hypothesize that they are important for freshwater mussel distributions. Water velocity and substrate type are known to influence mussel distribution and abundance (Vannote and Minshall, 1982, Strayer and Ralley, 1993). Both flow volume and groundwater recharge are related to water velocity. Additionally, the percentage of surrounding soil consisting of clay provides information regarding substrate type. The percentage of surrounding soil consisting of organic matter is important because freshwater mussels filter organic matter from the water column (Strayer et al., 1999), and presumably organic matter in the soil is related to organic matter in the water column.

The environmental data was converted to raster format in ArcGIS for Desktop Basic version 10.1 (esri.com). All rasters were sampled to achieve a common resolution of 100 m x 100 m and all rasters were in the NAD 1983 UTM Zone 15N projection using a geographic (XY) coordinate system with meters as the unit. Environmental layers were clipped in order to constrain them to lotic habitats. We did this by adding a 100 m buffer around water features (ponds, streams, rivers, canals, and dams), delineated by the NHDFlowline dataset (USEPA and USGS, 2005), and clipping the environmental layers to match the lotic buffer. These maps were used to make some general recommendations of locations of critical habitat for each species.

Models were validated using the test AUC, or the area under the operator receiving curve. AUC measures the probability that a randomly chosen presence site will be ranked above a randomly chosen pseudoabsence site (Phillips and Dudik, 2008). The test AUC represents the percentage of the pseudoabsence data with lower habitat suitability scores than the test data. Importantly, this model validation procedure is based on data points (test data) that were naïve to the model building process, and thus represent a form a ground-truthing of the models with independent data.

To quantify the relative importance of the individual environmental variables to the models, the fit of each full model was compared to reduced univariate models (Phillips, 2006). If an environmental variable accounted for most of the model fit when modeled by itself (as compared to the full model that was based on all the environmental variables), then the environmental variable was considered important in determining the varying habitat suitability of the landscape for that model (Phillips, 2006).

Model fit was measured with the gain statistic. Gain is a likelihood (deviance) statistic that measures the model performance compared to a model that assigns equal habitat suitabilities to all areas of the landscape. Taking the exponent of the final gain gives the (mean) probability of the presence sample(s) compared to the pseudoabsences. For instance, a gain of 3 means that an average presence location has a habitat suitability of e^3 = 20.1 times higher than an average pseudoabsence site. The test gain that is reported is the average gain of test data as compared to the pseudoabsence data.

Results and Discussion

Temperature

Temperature at 30 degrees caused three mortalities, while temperature at 35 degrees caused nine mortalities. At 30 degrees, mortalities started occurring after 11 days, but at 35 degrees mortality began instantly. In July and August, temperature of the Sabine River reaches 30 degrees most years. At 35 degrees, there was a 43% mortality rate (Figure 1.1). It is likely that current summer temperatures are causing mortality of mussels. Climate change would only exacerbate this effect.

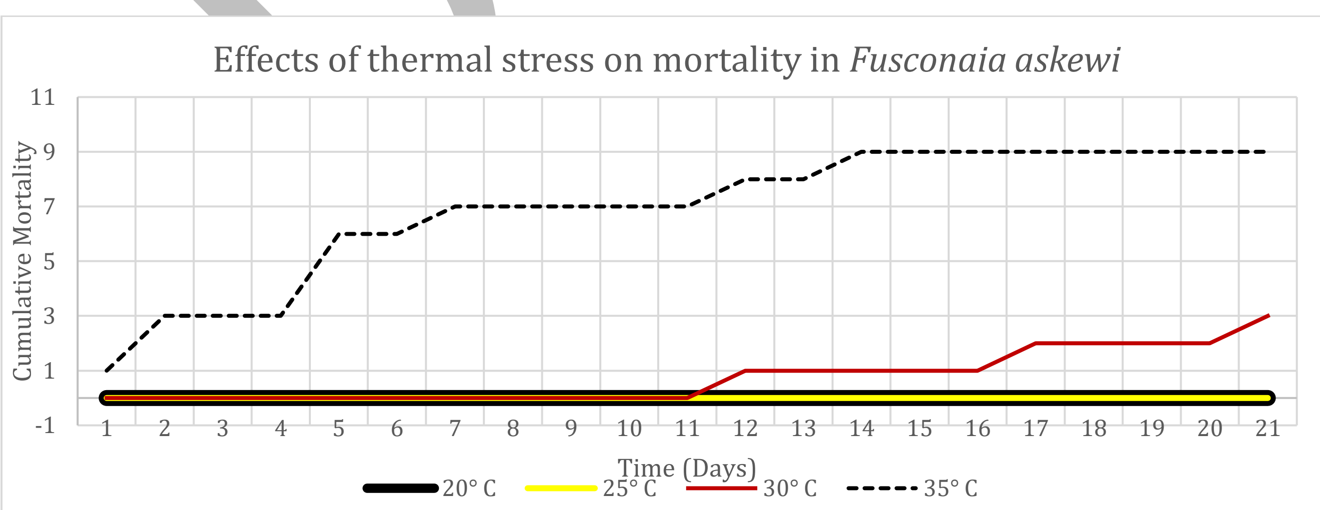


Figure 1.1 – Increased mortality is correlated with increased temperature in *F. askewi*.

There were no significant amounts of glycogen found in mussel tissues at temperatures below 35 degrees C. At 20, 25, and 30 degrees C, glycogen was not detected in more than one sample. At 30 degrees C, samples were found to contain glycogen with a mean concentration of 201.6 mg/L (range 137.3-268.3 mg/L). The wet weights of the excised tissues ranged from 80 to 130 mg per mussel and were diluted twenty-fold for determination of concentration along a standard curve. The aqueous standard curve was linear with R^2 values exceeding 0.96.

Siltation

Sedimentation experiments showed that burying mussels and 0.25 or 0.5 meters did cause mortality. Burying at 0.5 meters caused a 25% mortality over 96 hours (Figure 1.2). The mussels buried at 0.5 meters did not move at all. At 0.25 meters we did observe upward movement towards the surface. There was no mortality in the control animals. Movement was not noted until 48 hours into the experiment. The average movement after 48 hours was 11 cm, after 72 hours was an additional 10 cm, and after 96 hours was another 10 cm.

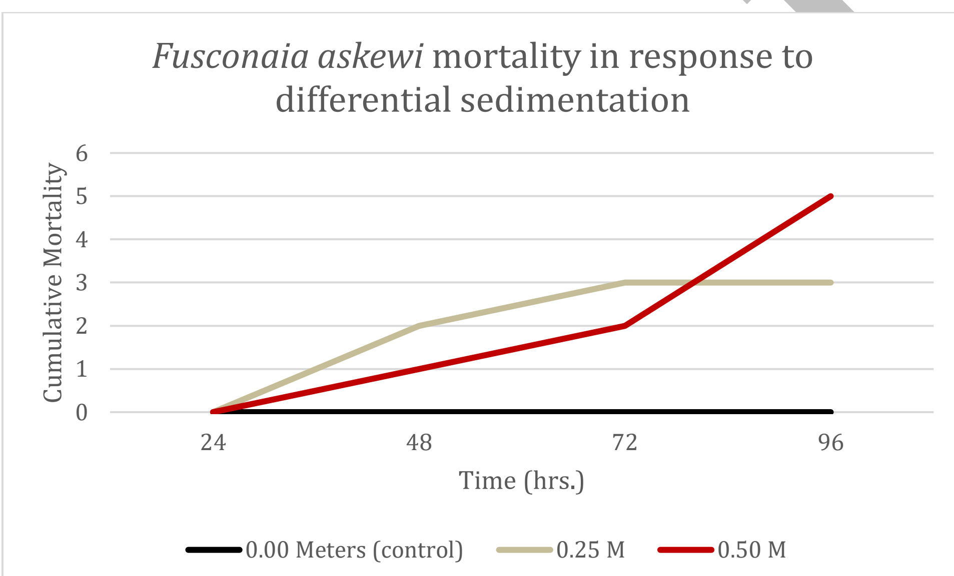


Figure 1.2 – Increased mortality is correlated with increasing depth of sedimentation over a 96 hour period.

Nitrogen

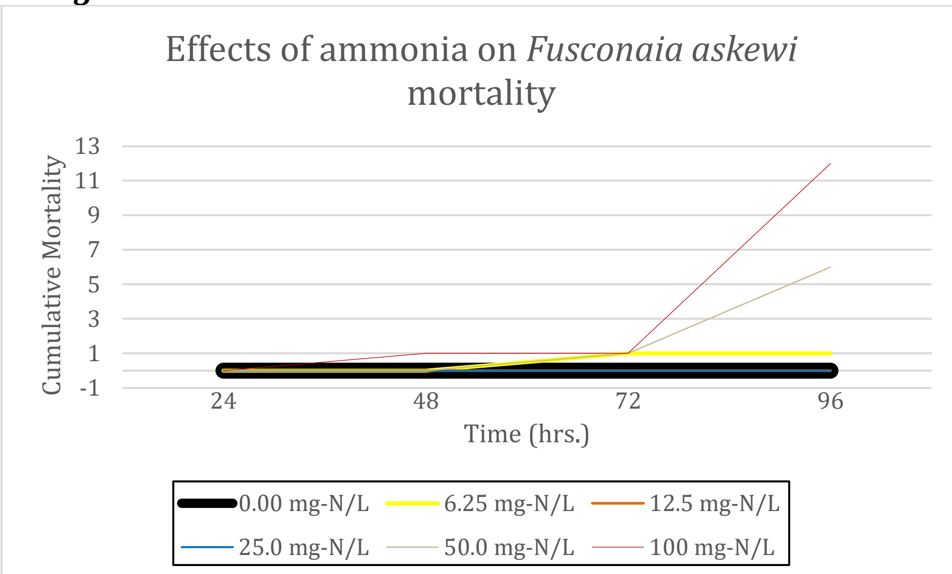


Figure 1.3.1 – Mussel mortality is positively correlated with increases in nitrogen concentration, over time.

Total mortality occurred at the highest concentration, and 50% mortality occurred at 50 mg-N/L (Figure 1.3.1).

Glycogen concentration decreased as ammonia concentration increased ($F = 2.43$, $p = 0.01$; Figure 1.3.2). As ammonia stress increased, mussels lost the ability to retain glycogen.

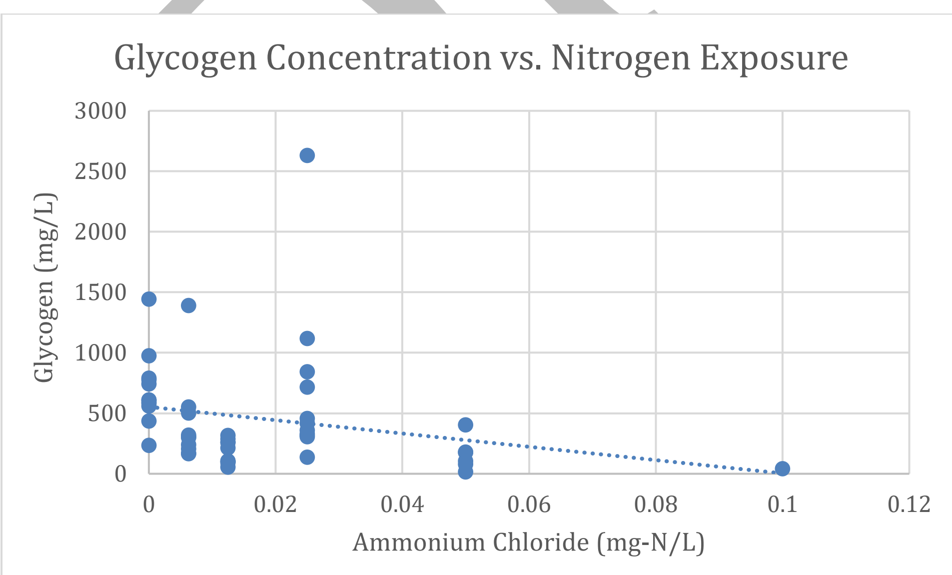


Figure 1.3.2. Glycogen vs. nitrogen exposure for Texas pigtoes.

Ecological Niche Modeling

The test AUC values for *F. askewii*, *P. riddellii*, and *P. amphichaenus* were 0.974, 0.979, and 0.990. Areas of highest suitability for *F. askewii* were: the upper Trinity River and its tributaries, as well as some tributaries of the lower Trinity River; the Sulphur River and its main tributary White Oak Creek; Cypress Creek and some of its tributaries; the upper Sabine River; the upper Angelina River; and the upper Neches River and some tributaries of the lower Neches River (Figures 1.4.1 and 1.4.2). Areas of highest suitability for *P. riddellii* were: a small segment of White Oak Creek; Cypress Creek and some of its tributaries; the upper Sabine River; the upper Angelina River; the upper Neches River and a tributary of the lower Neches River; and a segment of Attouyac Bayou (Figure 1.4.3 and 1.4.4). *P. amphichaenus* overall had a larger potential range size, as inferred from having more areas of suitable habitat. The areas of highest habitat suitability were: the Sulphur River and its main tributary White Oak Creek, as well as other tributaries; the upper Sabine River and its tributaries; the upper Angelina River and its tributaries; a segment of Attouyac Bayou; the Neches River and some of its tributaries (both the upper and lower Neches River); and the upper Trinity River and scattered tributaries of the lower Trinity River (Figure 1.4.5 and 1.4.6).

Volumetric flow rate was the most important contributor to the models of each species, as measured by the test gain of that variable when modeled alone as compared to the test gain of the full model. Stream velocity was the next most important contributor to the models for *F. askewii* and *P. amphichaenus*, but *P. riddellii* was different. For *P. riddellii*, there were also substantial contributions to the model from clay content, slope, organic matter content, and erodibility. Oil and gas development and chemical inputs had negligible influences on the models, suggesting that these variables are not important in determining the distributions of any of these three species (Table 1.4 and Figure 1.4.7).

Table 1.4. Test gains for the full model (first row) as well as models fit with only the one variable indicated (following rows), for each species (columns). The closer the test gain of the model with only that variable is to the test gain of the full model, the greater the contribution of that variable to the model.

	F. askewii	*P. riddellii*	*P. amphichaenus*
Full Model	2.691	3.0097	3.4149
Only Chemical Inputs	0.2126	-0.2705	0.2131
Only Recharge	0.3415	0.653	0.4222
Only Velocity	1.4598	0.7936	2.0704
Only Volumetric Flow Rate	2.5509	2.0577	2.9289
Only Oil and Gas	-0.0316	0.1571	0.0687
Only Sinuousity	0.3329	0.4819	0.7007
Only Available Water Capacity	0.3641	0.3172	0.5621
Only Bulk Density	0.1903	0.736	0.2255
Only Clay Content	0.5358	1.5211	0.3945
Only Erodibility	0.6155	1.2844	0.5039
Only Organic Matter Content	0.3303	1.2885	0.0593
Only Slope	0.8584	1.3748	0.6924

Figure 1.4.1. Habitat suitability map for Texas pigtoes in east Texas. Areas of warmer colors indicate higher habitat suitability.

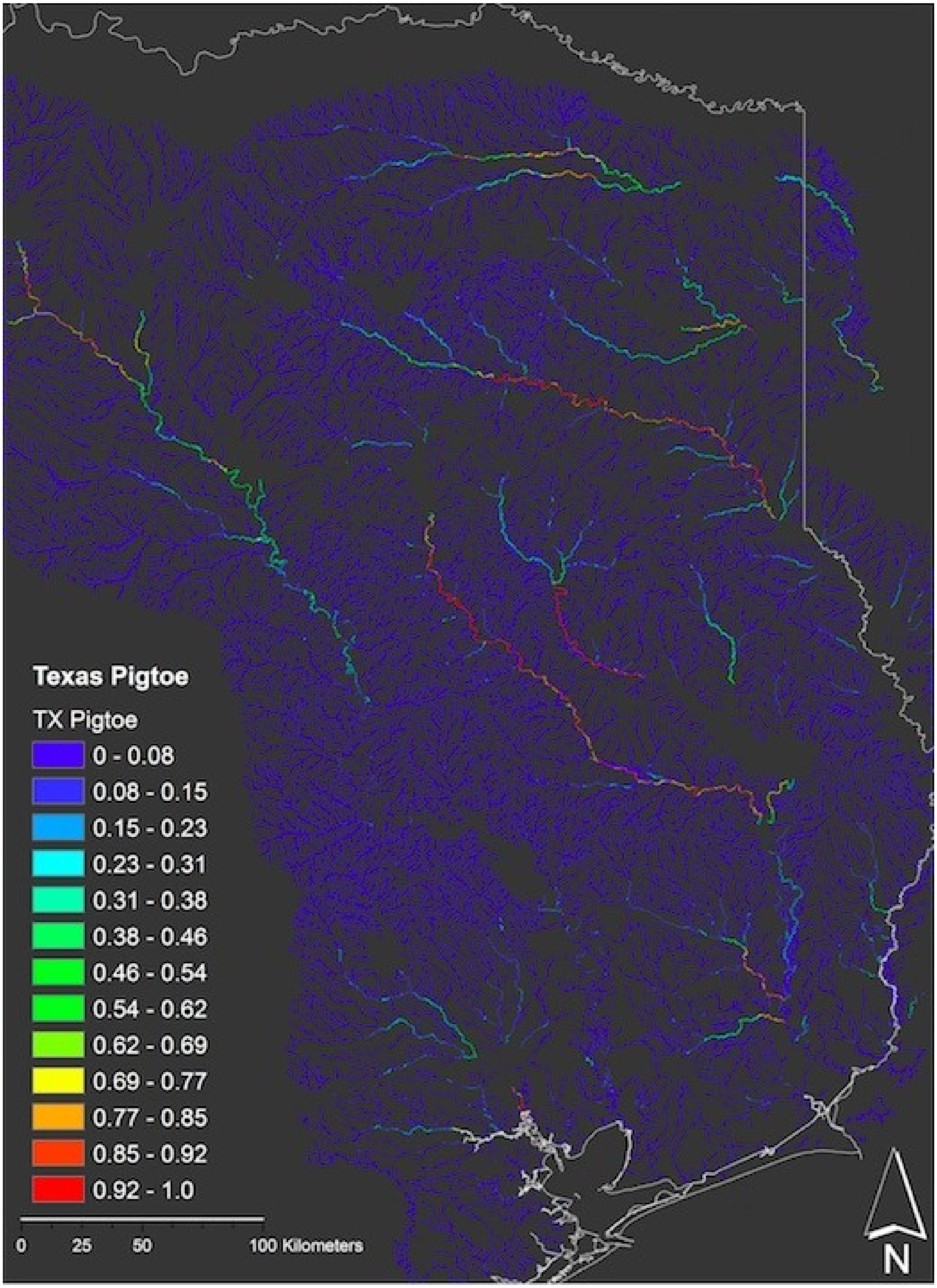


Figure 1.4.2. Habitat suitability map for Texas pigtoes in northeast Texas with stream names highlighted. Areas of warmer colors indicate higher suitability.

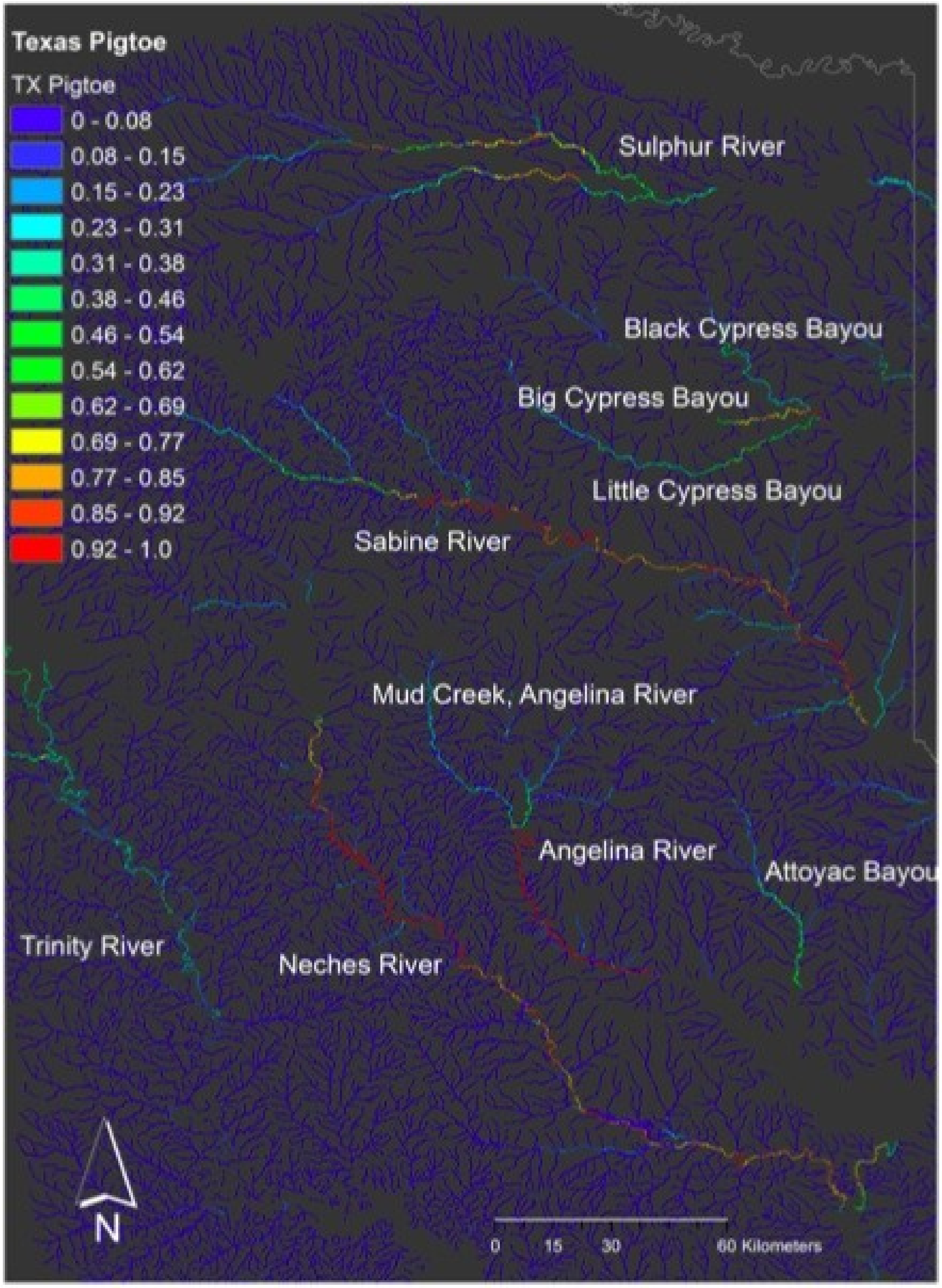


Figure 1.4.3. Habitat suitability map for the Louisiana pigtoes in east Texas. Areas of warmer colors indicate higher suitability.

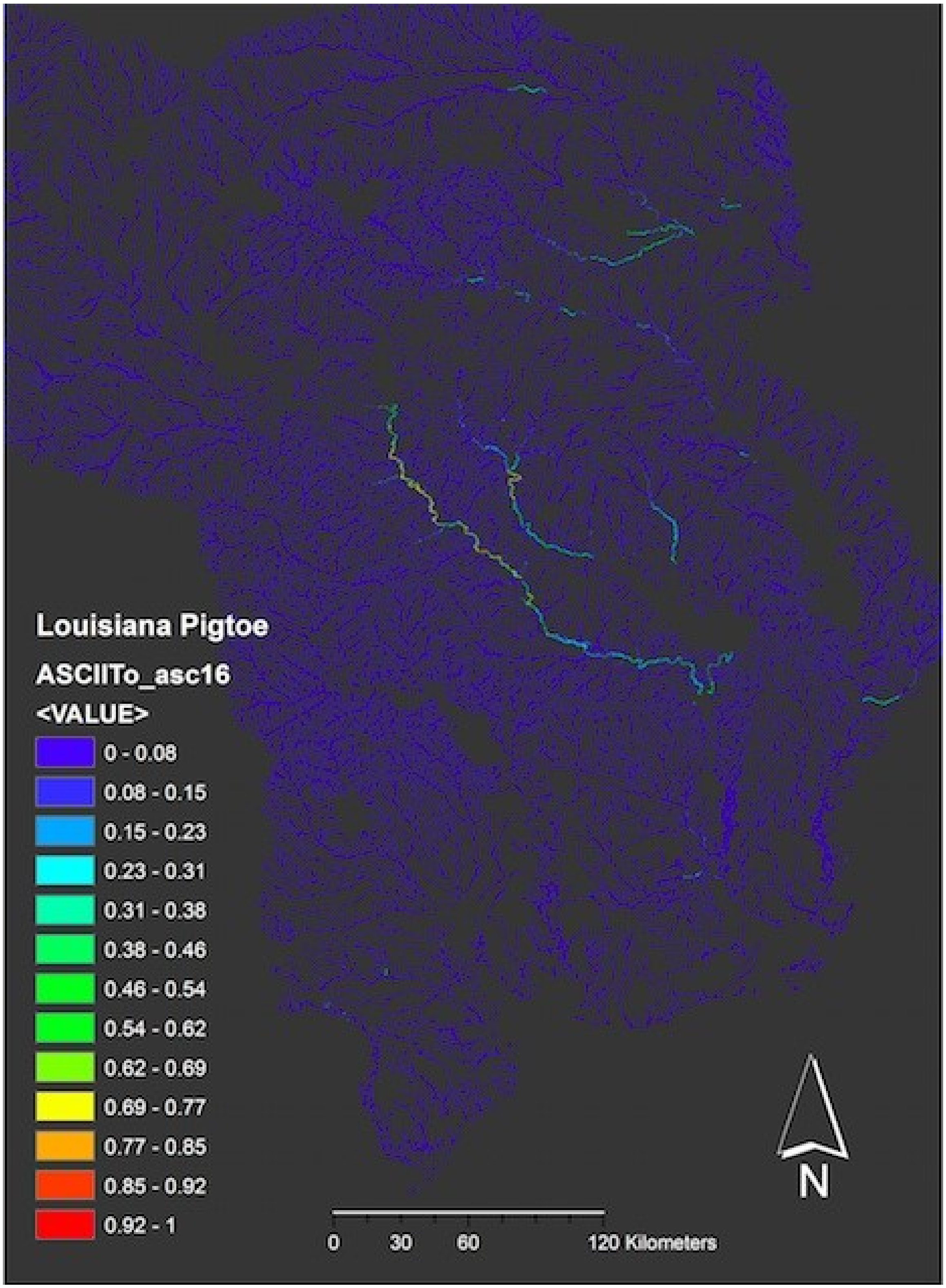


Figure 1.4.4 Habitat suitability map for Louisiana pigtoes in northeast Texas with stream names highlighted. Areas of warmer colors indicate higher suitability.

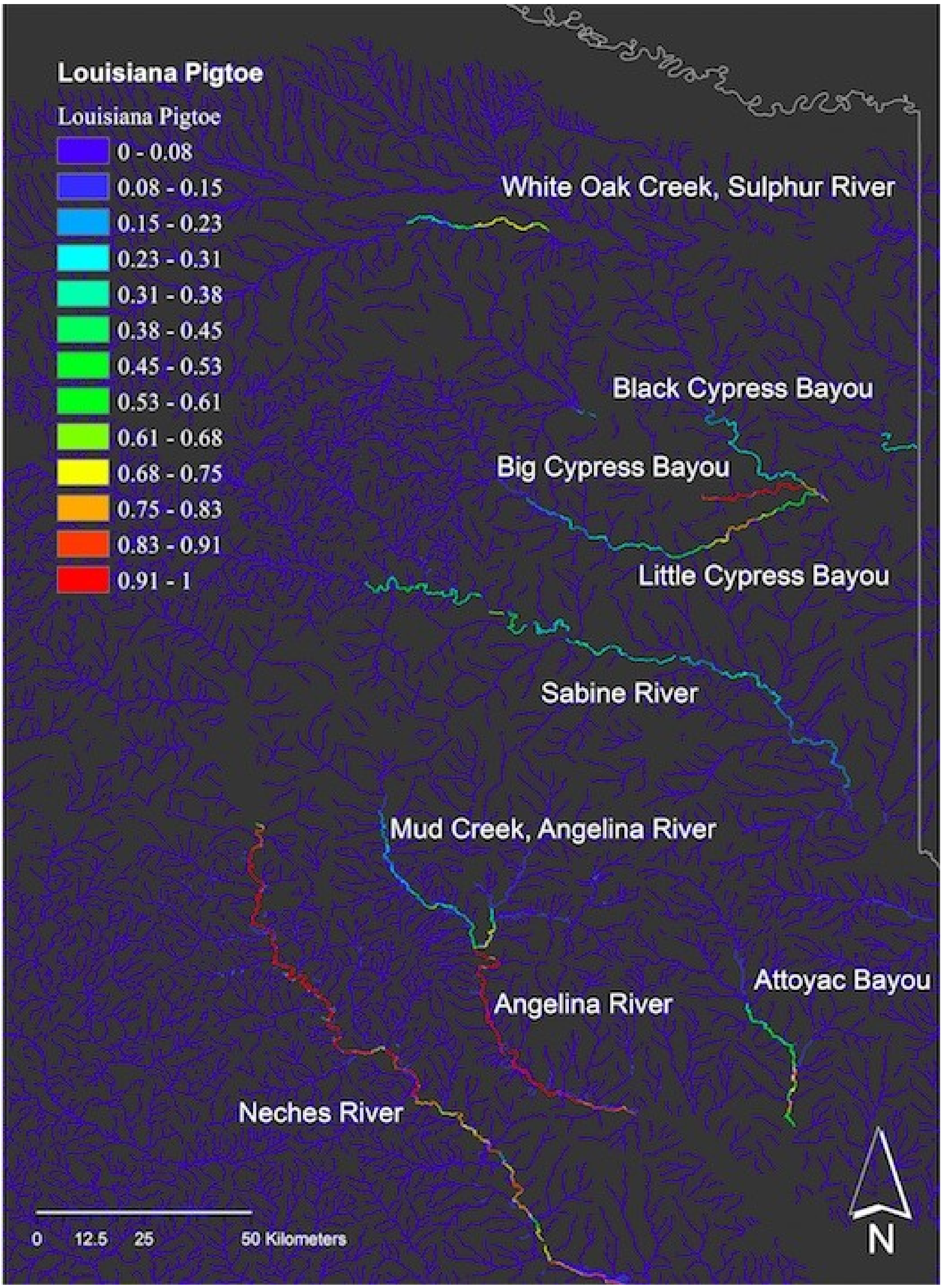


Figure 1.4.5. Habitat suitability map for Texas heelsplitters in east Texas. Areas of warmer colors indicate higher suitability.

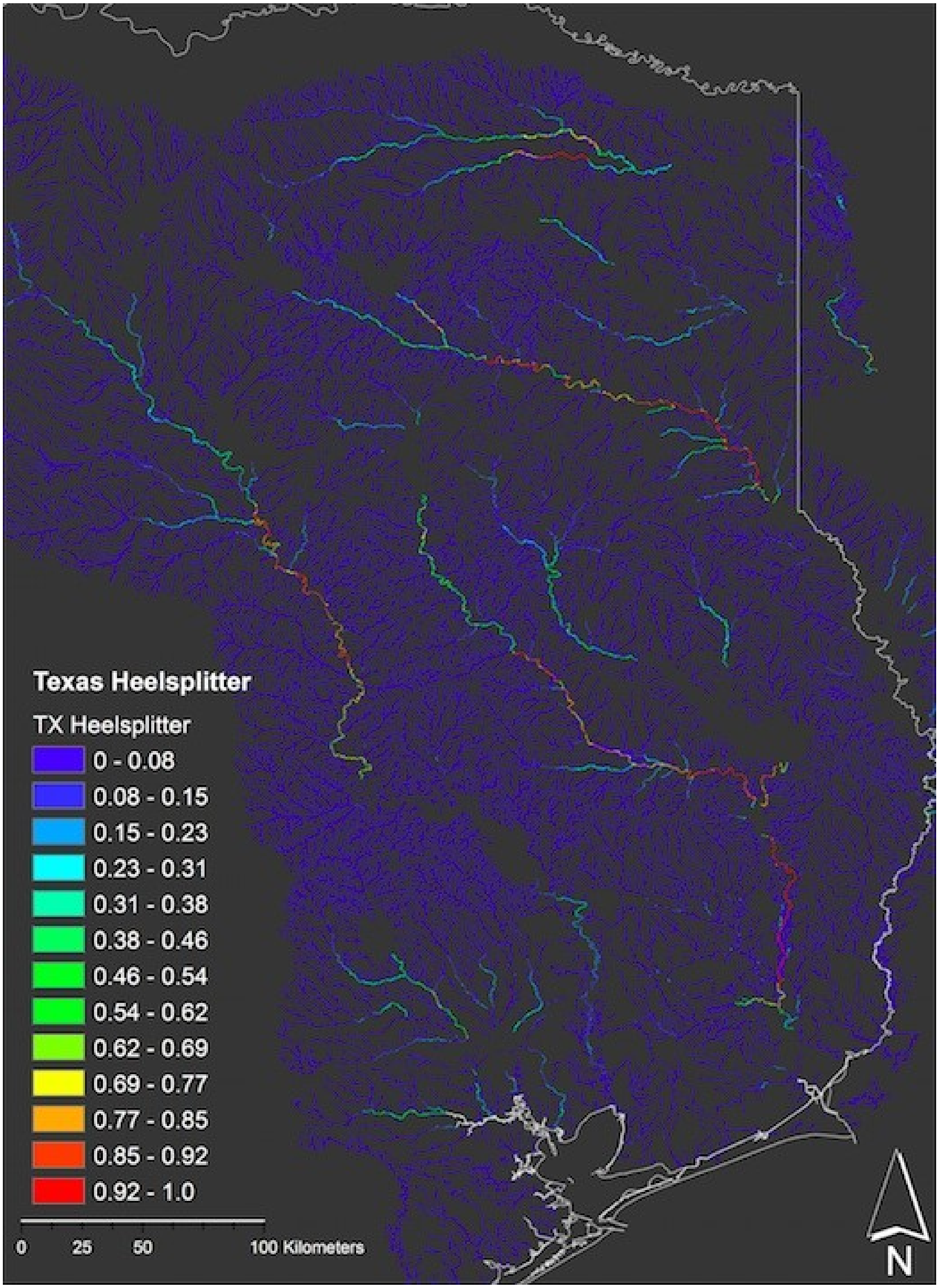


Figure 1.4.6 Habitat suitability map for Texas heelsplitters in northeast Texas with stream names highlighted. Areas of warmer colors indicate higher suitability.

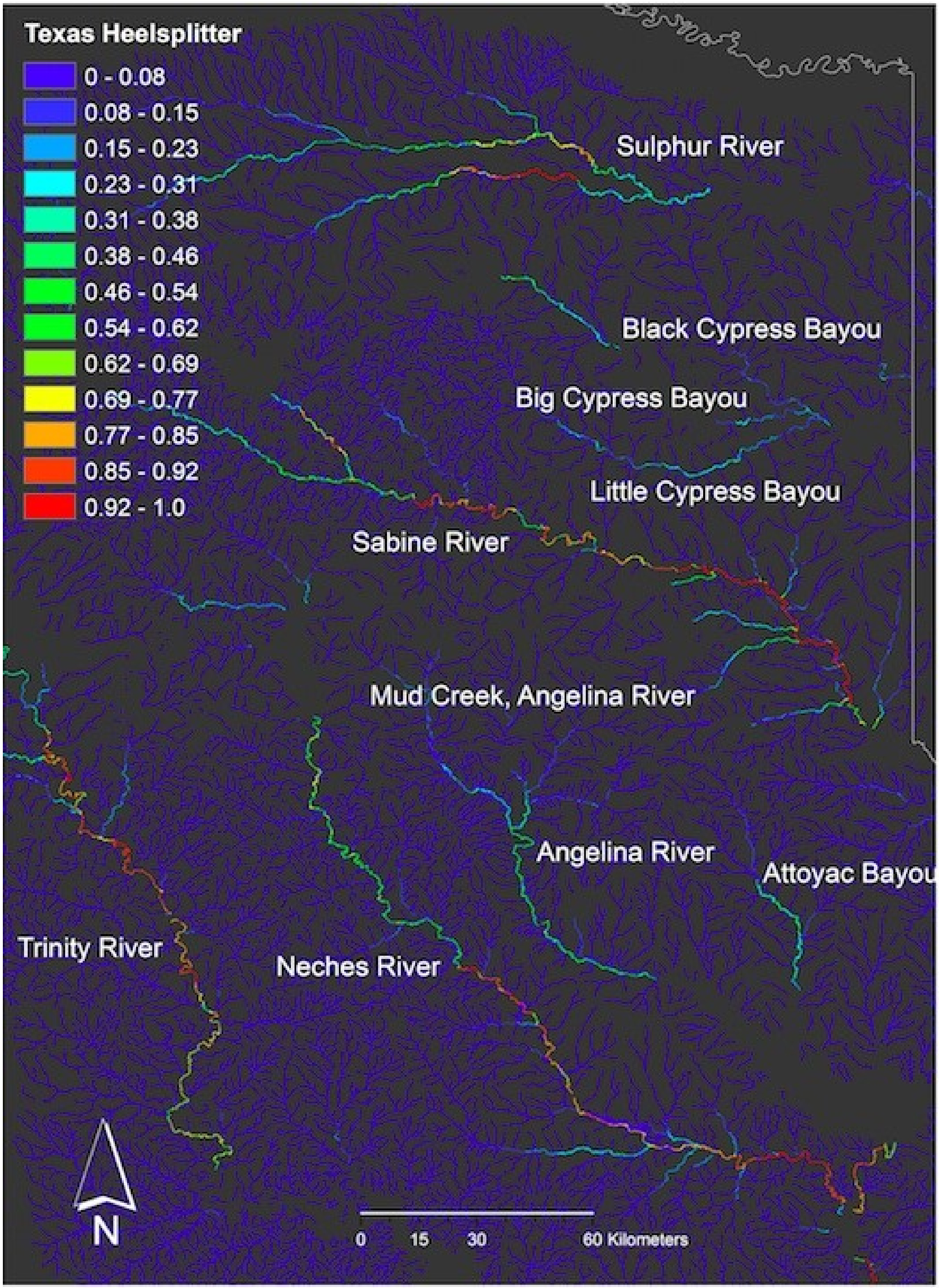


Figure 1.4.7. Habitat suitabilities as a function of the environmental variables for the three species. These “response curves” were generated from univariate models containing only one environmental variable at a time. The response curves for each species are in different columns. The response curves for different variables are in different rows. Each response curve shows habitat suitability (*y*-axis) as a function of the environmental variable (*x*-axis).

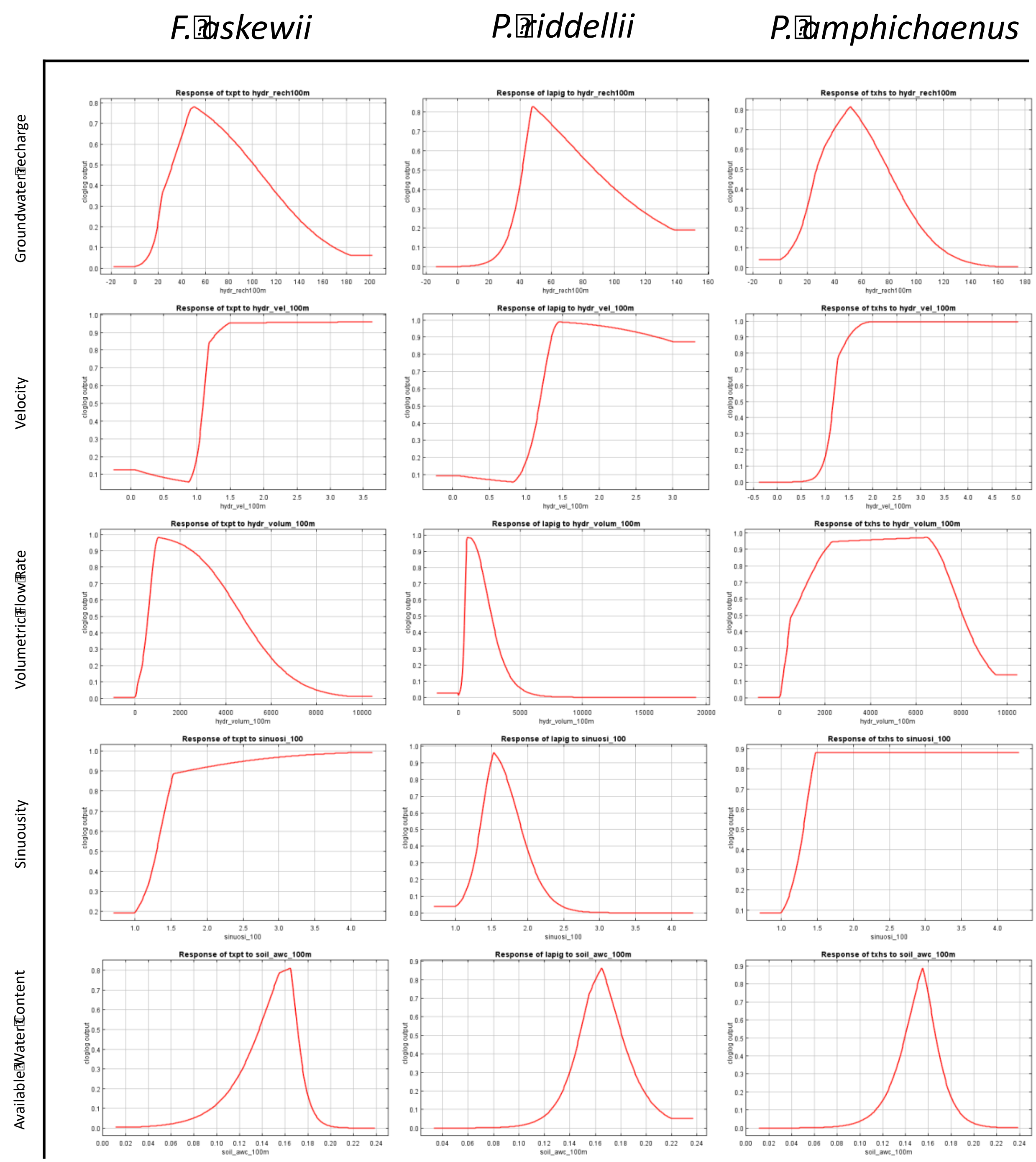
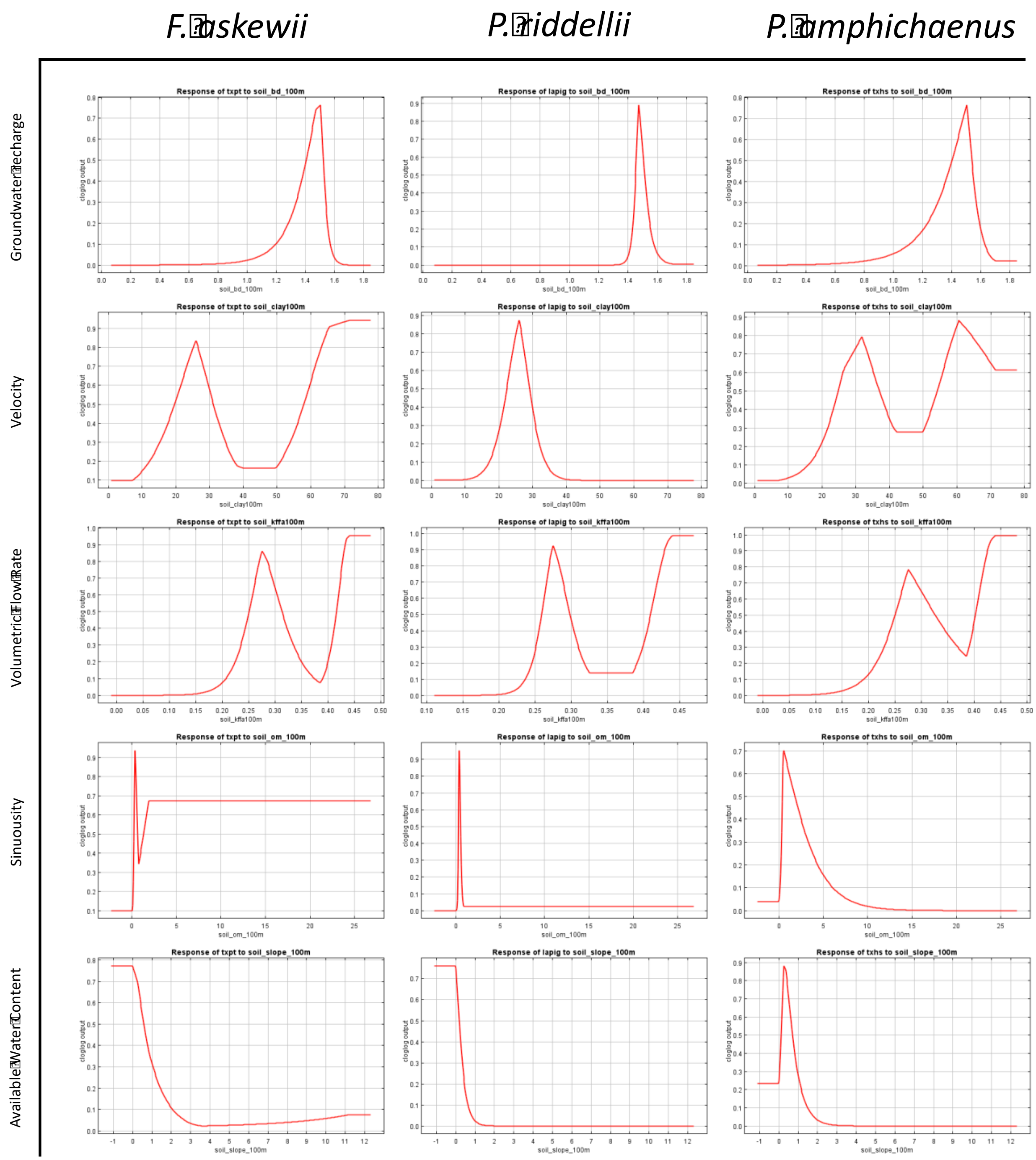


Figure 1.4.7 (Continued).



Task 2. Additional surveys to improve distributional information on *Fusconia*, *Pleurobema,* and *Potamilus amphichaenus* in East Texas.

Suitable habitat for freshwater mussels is dependent on the correct substrate and flow, which supply nutrients and a stable environment (Haag, 2012; Randklev, et al., 2015). Inadequate flow from lack of precipitation is the most critical environmental factor influencing mussel occurrence in central and west Texas. In east Texas the rivers are larger and rainfall is more consistent therefore these impacts are less relevant to unionids in the 6 river basins in east Texas. In east Texas environmental stressors are primarily related to landscape level problems such as proximity to dams, surrounding agriculture, and pollution from industries like oil extraction and urban wastewater releases. Current and historic distributions of these environmental stressors are important to understanding how these factors impact mussel species. In addition, these stressors are increasing as the population in Texas rapidly grows. For example, 26 new major reservoirs are proposed for Texas and oil and gas exploration is continuing to increase. We have been surveying the 6 major rivers in East Texas and have a good overall distribution for the 3 pigtoe species (*Fusconaia* and *Pleurobema*) and the Texas Heelsplitter (*Potamilus amphichaenus*. We produced MaxEnt maps with this data to show the suitable habitats for each species. However, two particular deficiencies were evident in our early surveys. They tended to be relatively close to access points such as the bridge boat ramps where we entered the rivers and only a few tributaries of the larger rivers had been surveyed. To produce better MaxEnt maps to evaluate the impact of the various environmental issues on these animals we conducted additional surveys stressing sites that would improve those distributions. These included:

1. 1179 additional surveys conducted in the summers of 2016 and 2017. 7579 live and 64 recent dead mussels of 29 species were recorded. Most sites for surveys were selected in areas where access was limited (We kayaked several miles from bridge access points on the Sabine River and the Neches River). We also kayaked up tributaries from the mainstem of both rivers. Tributaries had not been often surveyed in our previous work because of issues with obtaining landowner permissions at road crossings. A few tributaries where we had landowner permission were also surveyed.
2. We present both raw abundances for sites and catch per unit effort. This information is needed to better understand areas of potential for critical habitat designation.

Task 2.1. Additional Surveys

Methods

Sites were chosen from our distributional maps for *Fusconaia* and *Pleurobema* (pigtoes) and *Potamilus amphichaenus* (Texas Heelsplitter) further from our access points (several km from bridges) than in previous surveys. We also went to tributaries that were located on these rivers evident from Google earth. When we found those tributaries entering the mainstem, we paddle 40 meter upstream from the mainstem and conducted tactile surveys for 0.5 person hour. The shorter time period was chosen because the widths were often narrow and so the time required for adequate sampling was less than on the mainstem. If mussels were found, then an additional 0.5 person hour survey 40 meters up from the first site was then conducted. If any new species were encountered, we repeated the surveys upstream until no new species were found in two consecutive sites. Preference for reaches that contained multiple mesohabitats (riffles, runs and pools) were made when possible but most sites were shallow runs. Both live and recent dead mussels of the species of concern were recorded. At the entrance to each tributary one site upstream and another downstream on the mainstem were surveyed for one person hour. Geospatial information was recorded for each site. Raw abundance per site was recorded and a calculation of the number collected per person hour was made (CPU). Ecological niche modeling of mussel occurrence in reference to specific landscape impacts (i.e., distance from dams, number of oil rigs, area of agricultural land, etc.) was conducted. Mussel occurrence was compared relative to landscape effects and correlational analyses of these impacts to mussel distributions were produced. The MaxEnt map was compared to previous maps to determine if the sampling of tributaries had modified the results.

Results

1179 additional surveys were conducted in the summers of 2016 and 2017. Because only the upper Sabine and Neches Rivers had many permanently flowing tributaries, the surveys were concentrated in those rivers and their tributaries. Mainstream sites were selected based on maps of previous surveys with attempts to go further away from the bridge access points. A total of 42 named and unnamed creeks of the Sulphur, Sabine and Neches Rivers were surveyed. A total of 7579 live and 64 recent dead mussels of 29 species were recorded (Appendix). It was not possible to separate identification on the two *Fusconaia* species morphologically so all were recorded as Texas pigtoes. A total of 32 sites recorded no mussels, primarily small streams with significant amounts of organic debris from flooding.

Table 2.1.1. Abundances and number collected per unit effort (CPU) for 3 state listed species of freshwater mussel found in rivers in east Texas. Mean plus or minus one standard deviation and ranges are given. CPU is based on one person-hour. Most surveys were one person-hour in length.

Species	Abundance	CPU
Texas Pigtoe	9.33 ± 15.49 (1-80)	10.01 ± 15.58 (1-80)
Louisiana Pigtoe	3.18 ± 2.66 (1-13)	3.64 ± 3.38 (1-14)
Texas Heelsplitter	1.5 ± 1.38 (0-4)	1.5 ± 1.38 (0-4)

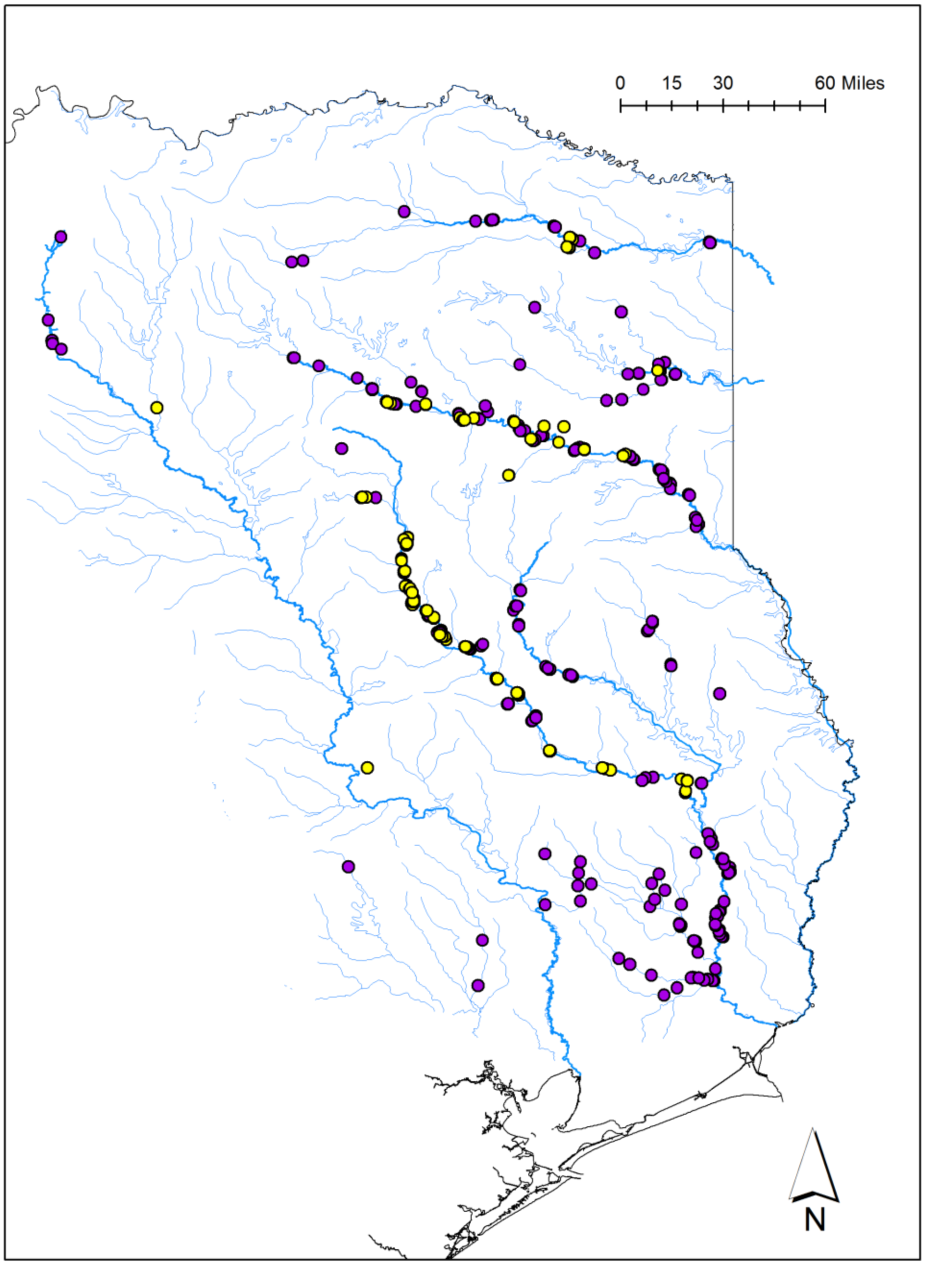


Figure 2.1.1. Map of sampling locations in East Texas. Points in yellow were collected on this project.

Discussion

Overall, the Texas pigtoe is one of the most common species of mussels in our surveys with over 9 recorded per person-hour. However, in the past we preferentially surveyed habitats that this species prefers, i.e. rocky riffles. Rocky riffles are the mesohabitat that is least common in east Texas rivers and is easily covered by bank erosion and siltation. When we surveyed backwater tributaries this species had one of the lowest encounter rates. The only sites were in a tributary that was actually an oxbow that returned to the river downstream.

The Louisiana pigtoe was much less common in our surveys with about 3 recorded per person-hour. It also is most common in riffles, primarily in the Neches River. However, some were recorded in larger backwater tributaries of the upper reaches. It is extremely rare in the Sabine River and was not found in any tributaries of that river.

The Texas Heelsplitter is one of the most rare of east Texas mussels and in these surveys only 1.5 per person-hour were recorded. However, it was found in one tributary that was full of leaves and debris. Its typical habitat is the sandy banks and backwater pools of the larger rivers so it may have a greater tolerance for low oxygen. The habitat of this species is much more abundant than riffles so why its numbers are so low may be a natural phenomenon. This habitat is not declining in our rivers.

Although a large number of tributaries were surveyed in this study, few had mussels and even fewer had any of the threatened species. Four tributaries had a total of 24 Louisiana pigtoes. Two tributaries had each one and two Texas pigtoes respectively. Only one Texas Heelsplitter was found in a tributary. Those tributaries with the pigtoes species were larger with less organic material. The Texas Heelsplitter was in a tributary with lots of litter and debris. Generally, tributaries were small and potentially ephemeral. Although some mussels can tolerate drying out the species of concern here require permanent water that flows.

Task 2.2. Surveys in Different Geomorphic Mesohabitats

Methods

This portion of the study consisted of 31 sites, located in reaches of the Upper Neches River between Lake Palestine and B. A. Steinhagen Lake. Sites were scouted via kayak and boat, guided by previously developed habitat suitability models, the presence of cretaceous rock as shown by ARC-GIS, and known locations of high abundance, state listed threatened species (Williams et al. 2013). All sampling was conducted at baseflow and was completed between July and September of 2016.

Field Sampling

Once a site was selected, it was visually assessed by two independent observers in order to delineate the stream segment into three mesohabitats; riffle, run, and pool. A riffle was defined as a segment characterized by a high point in the stream bed topography, faster flow, shallow depth, steeper water surface slope, and coarser bed material. A pool was defined as an area characterized by low points in stream bed topography, gentle water slope, slow flow, and finer bed material. A run was designated as the area directly upstream and downstream of a riffle that still contained faster flows, however it was of intermediate depth and had less surface turbulence. The Neches River is characterized by soft substrates such as sand and silt, and only sporadic areas of cobble and seldom boulder substrate. This leads to few areas that could be classified as riffles. Each site was sampled for mussels quantitatively through the excavation of 10, quarter-meter quadrats per mesohabitat unit that was delineated. This method of site determination lead to sampling 72 mesohabitats across 31 sites.

A stratified random sampling method was used that employed one random start in each of the three mesohabitats (riffle, run, and pool), resulting in 10 samples being conducted in each mesohabitat. Random coordinates were generated initially to determine the start point in each mesohabitat. Subsequently, the distance to the next sample unit was calculated using the following formula and randomly generated directions: $d = \sqrt{\frac{(L*W)}{(n/k)}}$ where d is the distance to next sample, L corresponds to length of study site, W is the width of study site, n is the number of samples taken, and k represents the number of random starts (Strayer and Smith 2003). Each quarter-meter quadrat was assessed through a tactile search, and all exposed mussels were collected. Once complete, the entire quadrat was hand excavated to an approximate depth of 15 centimeters (cm), and all harvested material were processed and washed through a sieve to ensure all mussels within the sample area were collected.

Habitat Variables
Habitat variables were collected in each mesohabitat by running a transect through a representative area in each unit and collecting measurements at three points along the transect. The habitat variables collected in each unit included flow, depth, and bank angle. A flow meter (Flo-Mate™ model 2000) was used to collect flow at both banks and in the thalweg perpendicular to flow, while depth was taken simultaneously. The angel to the top of each bank was measured from the surface of the water, at each point along the transect, using a clinometer. Within each unit a substrate sample, approximately 1 kg, was taken in order to conduct an in-lab sieve analysis to determine percent composition of substrate for each unit.

Substrate Analysis
Substrate samples were dried in ovens at 105° Celsius for 24 hours to remove all water. In order to determine percent composition of each substrate class contained within each mesohabitat at each sample site, a sieve analysis was conducted on each dried substrate sample. The sieving process involved thoroughly shaking samples through a series of sieves that have openings of progressively smaller size from top to bottom. The samples were loaded into the top sieve and a shaker machine was used to rigorously agitate the sample. Samples were run for 10 minutes which allowed sufficient time for the sample to be completely separated into each sieve with a specific sediment size (Das 1998). The substrate in each sieve could then be weighed and the percent composition of each substrate class could be determined by dividing the weight of total substrate in each class by the total sediment sample size. Substrate classes were defined as follows: silt, < 0.063 millimeters (mm); sand, 0.063-2 mm; gravel, 2-64 mm; cobble, 64-256 mm; boulder, 256-330mm; bedrock, > 330 mm (Minshall 1984). Dominate substrate was defined as the

substrate class with the highest overall percentage in each unit, while subdominate substrate was considered the substrate class with the second highest percentage composition. If a single substrate class comprised more than 95% of the sample, as was often the case, dominate and subdominate substrate were considered to be the same.

Hydraulic Variable Calculations

We calculated four different hydraulic variables for each mesohabitat that was sampled. Shear stress, shear velocity, relative shear stress, and critical shear stress were calculated according to the parameters laid out by Allen and Vaughn (2010) (Table 2.2.1). Shield's parameter (Θ_c) was assumed to be 0.065 which is associated with closely packed substrate that contains smaller material similar to the substrate at my sample sites (Gordon et al. 2004). Flow and depth variables were measured in stream. D_{50} corresponds to the particle size at which 50% of the substrate sample is smaller than. Bed roughness coefficient (k_s) is estimated by multiplying D_{84}, the particle size at which 84% of the substrate sample is smaller than, by 3.5 (Gordon et al. 2004). The percent passing through each sieve was graphed as a function of particle size and used to determine D_{50} and D_{84}. Shear velocity is also known as friction velocity and gives insights about the velocity profile near the substrate (Statzner et al. 1988). Shear stress is referred to as the amount of force (dynes/cm^2) being exerted on the substrate at the substrate-water interface due to the shear velocity (Statzner et al. 1988). The amount of force per square cm (shear stress) required to initiate movement of 50% of the substrate within a sample is referred to as critical shear stress (Gordon et al. 2004). Relative shear stress is a ratio of shear stress to critical shear stress and can be used as an index of the stability of the substrate (Morales et al. 2006).

Three-way Log Linear Contingency Tables

In order to investigate habitat associations based on mesohabitats and environmental characteristics or hydraulic variables, three-way log-linear contingency tables were developed using the "xtab" function in R (R Core Team 2013). Contingency tables are a type of matrix that takes abundance data and places it into categories based on certain variables (i.e., mesohabitat and subdominate substrate for a certain species) and allows for the investigation of relationships between these variables. Numeric, environmental characteristics were placed into three different value bins by creating a low, median, and high category. Each category represents one third of the total range of values that were recorded during the field sampling, except for entrenchment ratio. The bins in entrenchment ratio correspond to slightly, moderately, and highly entrenched values based on Rosgen (1994). Residual analysis was conducted in order to ensure no outliers skewed the results. Contingency tables were developed in each mesohabitat, for each environmental or hydraulic characteristic resulting in ten, three-way contingency tables for each species (Table 2.2.5). In order to have a large enough sample size to satisfy the assumptions of log-linear contingency tables, a species had to have more than 15 individuals collected resulting in ten contingency tables for 17 species.

We were interested in determining if the distribution of mussels in each mesohabitat, with certain environmental or hydraulic characteristics, was random or diverged from what we would expect to see at random indicating some other mechanism driving distribution. We applied a χ2 test to each table within the output. This was done to determine if each species were occurring in that combination of mesohabitat and environmental characteristic at random or if the observed counts deviated from expected. Because this analysis was a multiple test procedure, the p-values were corrected using a Bonferroni correction. A Bonferroni correction requires the critical p-value (α) to be divided by the number of comparisons being made in order to account for multiple tests being performed, and to guard against Type I error (Holm 1979, Hockberg 1988). The number of species being assessed for each environmental variable represented the number of comparisons being made.

Entrenchment Ratio

Entrenchment ratio is defined as the vertical containment of the river, and the degree to which it is incised in the valley floor. It was calculated on the reach level, as it is assumed it does not vary significantly across a single reach. The ratio is calculated as follows: $ER = \frac{Flood\ prone\ width}{Bankfull\ width}$. Arc-GIS was used to derive these values from the associated sampled reaches.

Canonical Correspondence Analysis

A canonical correspondence analysis (CCA) was performed in order to determine if the community of mussels that were sampled grouped based on the set of environmental characteristics that were sampled in each mesohabitat. A CCA is a multivariate method of analysis that takes multiple variables measured on the same individuals and explores the relationship between all variables and the individual (Vaughn and Taylor 2000). The analysis was run in CANOCO 4.5 and illustrated in CANODRAW (Figure 2). A Pearson correlation analysis was performed to correct for spatial autocorrelation. A correlation score above a threshold of 0.75 resulted in one variable being removed from analysis

Results

Our sampling for this portion of the project yielded 24 total species and over 1,500 total individuals. We were able to characterize a wide variety of mussel beds from dense, gravel-based riffles to sparse, sand filled pools. In total, 72 units were sampled, with flow, depth, bank angle, substrate, shear stress, shear velocity, critical shear stress, and relative shear stress determined for each unit (Table 2.2.2). Entrenchment ratio was determined for each sample site. The in-lab sieve analysis yielded dominant and subdominate substrate for each unit, as well as D_{50} and D_{84} values to be used in the hydraulic variable calculations. The range of calculated hydraulic variables is presented to illustrate typical values for an East Texas river (Table 2.2.3).

We developed ten three-way log linear contingency tables, one for each environmental or hydraulic variable, for all 17-species resulting in 170 total tables. Few collections occurred in units that were within the high category of the hydraulic variables, and there were no significant associations with hydraulic variables in these categories (Table 2.2.6). Shear velocity associations were distributed between both the low and medium category, while all shear stress and critical shear stress associations were in the low category (Table 2.2.6). Only two species were found to have associations with relative shear stress, which describes substrate stability. Threeridge (*Amblema plicata*) associated with stable substrates, and Texas Pigtoe (*Fusconaia askewi*) associated with areas with loose substrate (Table 2.2.6). Concerning entrenchment ratio, Texas Pigtoe (*Fusconaia askewi*) was found to associate with highly entrenched run areas, while Washboard (*Megalonaias nervosa*) was found to associate with slightly entrenched, pool areas (Table 2.2.6). All associations within dominate substrate were for sand substrate, which is explained by east Texas being characterized by soft substrates such as sand (Table 2.2.6). Subdominate substrate associations were found to be primarily for gravel, which increases bed stability (Table 2.2.6). Fawnsfoot (*Truncilla donaciformis*), Pistolgrip (*Tritogonia verrucosa*), Threeridge (*Amblema plicata*), and Western pimpleback (*Quadrula mortoni*) all exhibited associations with subdominate gravel substrate (Table 2.2.6). While many contingency tables failed to have an adequate sample size, the analysis yielded multiple significant outputs (Table 2.2.6). For example, the state listed mussel Louisiana Pigtoe (*Pleurobema riddellii*) had significant values ($p<0.05$) for average flow ($p<0.00$), average depth ($p<0.00$), shear stress ($p=0.02$), and shear velocity ($p=0.02$) (Table 2.2.4). While many species, like Louisiana Pigtoe, had significant values in multiple categories, some only had significance in one or two categories. For example, Gulf mapleleaf (*Quadrula nobilis*), only yielded significance in average depth ($p<0.00$) (Table 2.2.4).

Canonical correspondence analysis identified a significant relationship between species distribution and environmental and hydraulic variables ($p=0.002$) (Figure 2.2.2). The CCA was subjected to a Monte Carlo procedure to determine if the species data and environmental variables were associated, and was run 500 times. The first two axes of the CCA explained 21.8% of the variation across all the described environmental and hydraulic variables (Eigenvalue Axis 1 = 1.608; Axis 2 = 0.348). The length of the arrow on the CCA increases with increasing importance, and the proximity of a species to an arrow indicates the strength of relationship. Subdominate substrate is the longest arrow, indicting it is the most important environmental variable (Figure 2.2.2). Units with no species collections, labeled as "none", were analyzed and found to be highly associated with deep pools (Figure 2.2.2). Bankclimber (*Plectomerus dombeyanus*) was also found to be highly associated with pool habitats (Figure 2.2.2). Many species were found to be positively associated with entrenchment, represented by slightly entrenched stream segments, and included the Texas state-listed species, Southern Hickorynut (*Obovaria arkansasensis*) (Figure 2.2.2). Pistolgrip (*Tritogonia verrucosa*) was among the assemblage associated with riffle habitat (Figure 2.2.2). Louisiana Pigtoe (*Pleurobema riddellii*) was highly associated with run habitat (Figure 2.2.2). Texas Pigtoe (*Fusconaia askewi*) was found to be negatively associated with entrenchment ratio, which is represented by highly entrenched areas of the stream (Figure 2.2.2).

Discussion

Gaining a better understanding of freshwater mussel habitat requirements is essential to determining how to manage the declining populations of this fauna (Annie et al. 2013, Layzer and Madison 1995). The goal of this study was to investigate the importance of hydraulic variables and entrenchment ratio on mussel assemblage and distribution within a stream. We also were attempting to implement a mesohabitat sample scheme, and determine if there were associations based on these units, and based on certain environmental characteristics within these units. Previous studies have examined distribution at a reach or microhabitat scale; however, very few researchers have considered distribution and habitat association at a mesohabitat scale (Otsby et al. 2014, Hastie et al. 2000). Our analyses suggest that a species can be a habitat generalist, riffle, run, or pool associate, connectivity to the floodplain (entrenchment ratio) is associated with species assemblage, and that hydraulic variables may determine where in a stream a mussel can be found. When all this information is considered it suggests detailed habitat associations, and insights into where a species can occur for a range of species in the Neches River.

Through sampling 72 unique, mesohabitats we were able to characterize a wide variety of the available habitat for freshwater mussels in the upper Neches River. The log-linear contingency table analysis allowed us to determine if a species is being randomly distributed in the available mesohabitats with the environmental variable of interest, or if there is an association present (Table 2.2.6). While many species did not exhibit any association, and were randomly distributed in regards to certain environmental variables, many species did exhibit these associations. Using these data we were able to make inferences about what the critical habitat is for certain species. Flow, depth, bank angle, and substrate all appear to be positive mechanisms for some species. These species also demonstrated an affinity for certain mesohabitat types, and occurred in them significantly more than other units. These habitat associations give insights into what stream habitat is important to certain species of mussels. For example, Louisiana pigtoe (*Pleurobema riddellii*), Pistolgrip (*Tritogonia verrucosa*), Southern mapleleaf (*Quadrula apiculata*), and Threehorn wartyback (*Obliquaria reflexa*) were all found to be associated with runs that had flows between 0.44 and 0.66 m/s^2. Washboard (*Megalonaias nervosa*) were found to be associated with deep pools that had depths between 1.12 and 1.68 meters.

When we investigated the association of mussels in regards to hydraulic variables (shear stress, shear velocity, critical shear stress, and relative shear stress) all species with a significant preference for shear stress, critical shear stress were in the low category (Table 2.2.6). Texas Pigtoe (*Fusconaia askewi*) was shown to be associated with runs that have a relative shear stress between 0.9 and 1.97 indicating that it does not need to be in areas of high bed stability. A mix of low and medium shear velocity preferences were found for various species (Table 2.2.6). Coupled with little to no records of mussel collection for units that fell into the high category, these results suggest that mussels have specific requirements concerning hydraulic pressures and do not exist in areas above a certain threshold. This evidence indicates that in these high shear stress areas, even at base flow, freshwater mussels cannot stay embedded and physically cannot exist there. This is an example of freshwater mussel distribution being impacted by negative censoring. Riffle, runs, and pools are defined and delineated based on observations concerning flow, depth, and substrate. Each of these values plays an important role in calculating hydraulic variables. As flow increases there will be a correlated increase in the amount of force being generated on the substrate-water interface (shear stress). Taking into consideration this relationship between mesohabitat and hydraulic variables, it can be inferred that mesohabitats are a reliable surrogate for hydraulic variables at base flow and can be used to determine these areas that certain species cannot tolerate.

Strayer (1999) conducted an experimental study to find flow refugia within a stream and attempted to determine if mussel populations were utilizing these areas. He found some evidence of mussels inhabiting areas that were considered flow refugia. More recent studies have implemented specific metrics (such as shear stress and critical shear stress) in order to quantify how much force a specific area of a stream is experiencing (Morales et al. 2006, Allen and Vaughn 2010, Statzner et al. 1988, Gordon et al. 2004). One study in east Texas attempted to develop shear stress measures for the Neches River, however the values were measured on a reach level (Troia et al. 2015). I have developed a baseline of the ranges that hydraulic variables can exhibit in each mesohabitat, within a river dominated by sandy substrates. Specifically, using the relative shear stress metric, one can start to understand the substrate stability requirements of certain species, and what kind of hydraulic stress they tolerate.

Previous research has shown that in-stream environmental variables explain about 30% of the variation in freshwater mussel distribution while, fish hosts, and landscape level variables explain the remainder (Haag 2012, Mcrae et al. 2004). The results of the canonical correspondence analysis (CCA), explained approximately 22% of the variation in mussel distribution with my set of environmental variables. Grouping suggests that certain species associate with either riffle, runs, or pools (Figure 2.2.2). It also showed that subdominate substrate is the most important environmental variable. Considering that most of the Neches River is characterized by sandy substrates, having areas with large subdominate substrate, such as gravel or cobble, will increase bed stability offering higher quality habitat. Further examination of the CCA showed that some species occur close to the node, and therefore are not driven by these environmental variables. Considering this independence from these environmental variables, it is reasonable to conclude that they are habitat generalists. For example, this group of habitat generalists included Western Pimpleback (*Quadrula mortoni*), a species that was ubiquitous across sample sites in the upper Neches River. Considering the high abundance of Western Pimpleback's that were collected, it is possible that being a habitat generalist allows a species to inhabitat a wider range of habitats and experience higher abundance.

Another set of species grouped around entrenchment ratio, indicating they associate with areas of slight entrenchment. Because of the increased connection to the floodplain in these areas, shear stress values during high flow events will be similar to those at base flow. Our data indicate that the species assemblage shifts with changes in entrenchment ratio, indicating a high conservation value on areas with slight entrenchment (Figure 2.2.3). Southern Hickorynut (*Obovaria arkansasensis*), a Texas state-threatened species, is only found in the few areas that exhibit slight entrenchment on the Neches River. The other species that occur in the slightly entrenched areas of the stream include: Threehorn wartyback (*Obliquaria reflexa*), Threeridge (*Amblema plicata*), Bleufer (*Potamilus purpuratus*), and Gulf mapleleaf (*Quadrula nobilis*). A single species, Texas Pigtoe (*Fusconaia askewi*), was associated with areas of high entrenchment. Areas of high entrenchment have been shown to be associated with less healthy stream segments. This can be because of higher rates of erosion, little to no connection to the floodplain, reduced benefits of riparian vegetation, and overall declining quality in habitat (Ward et al. 2003).

Louisiana Pigtoe (*Pleurobema riddellii*), a mussel of conservation concern within Texas, needs active management to avoid continued population declines. The framework laid out in this report, allows the determination of what habitat this species associates with. These data suggest that it prefers runs, with a subdominate mix of gravel, flows between 0.44 m/s^2 and 0.66 m/s^2, depths between 0.57 and 1.12 meters, and stream segments that have shear stress values and shear velocities less than 8.43 dynes/cm2 and 1.67 cm/s respectively. Our data suggest that important habitat for Texas Pigtoe (*Fusconaia askewi*) includes highly entrenched runs that have flow rates between 0.22 and 0.44 m/s2 and depth falling in the range of 0.57 and 1.12 m. It prefers areas with shear stress' less than 8.43 dynes/cm2, shear velocity's less than 1.67 cm/s, and critical shear stress values less than 43.9 dynes/cm2; however, it can tolerate relative shear stress values that fall in the medium class between 0.9 and 1.97. Morales et al. (2006) developed the relative shear stress index as a way to determine the stability of the substrate. Values over 1.0 are considered areas with loose substrate while any value under 1.0 is considered stable. Considering Texas Pigtoe associates with highly entrenched runs and prefers areas that have less stable substrate, it can be inferred that substrate stability is not an important factor for this species. It has adapted, maybe through effective burrowing behavior, to remain embedded in these less than ideal areas.

As more freshwater mussels are receiving elevated conservation status, and waterways in North America continue to experience declining quality, the need for active management becomes more important. Through this research, we have been able to develop a framework that will allow managers to examine what the critical habitat for certain species may be, and identify reaches of streams that have unique assemblages, high quality habitat, and favorable in stream habitat. As more states start to develop recovery plans for their imperiled freshwater mussels this information on habitat will be vital.

Conclusion

This study investigated freshwater mussel distribution and habitat associations through sampling mussel beds at a mesohabitat scale, calculating hydraulic variables, and entrenchment ratio. Our protocol has resulted in a framework that will help determine the areas of the stream that are preferable to a species both in terms of negative and positive mechanisms. Our results indicate that some species of mussel's associate with only one of the three mesohabitats, can be generalist, or occur only in areas of certain entrenchment. We have shown that species assemblage changes in areas of the stream with slight entrenchment. Evidence indicates that mesohabitats can be used as surrogates for indices of flow refugia at base flow because depth, flow and substrate drive their delineation. The analysis of the data through log-linear contingency tables suggest that some species are associated with certain mesohabitats, environmental variables, and areas of lower hydraulic stress. We were able to develop some initial descriptions of the types of mesohabitats that may be important to some species. In order to determine the validity of sampling at a mesohabitat scale and the development of these habitat association, this sampling scheme and framework needs to be repeated across systems to determine if the findings are similar in other watersheds. As freshwater mussel populations continue to decline globally, determining their basic habitat requirements will continue to be important and this may be a valuable tool in that effort.

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Figure 2.2.1. The upper Neches River in east Texas. Red dots represent the 31 sample sites. Each site was delineated into the available mesohabitats (riffle, runs, or pools).

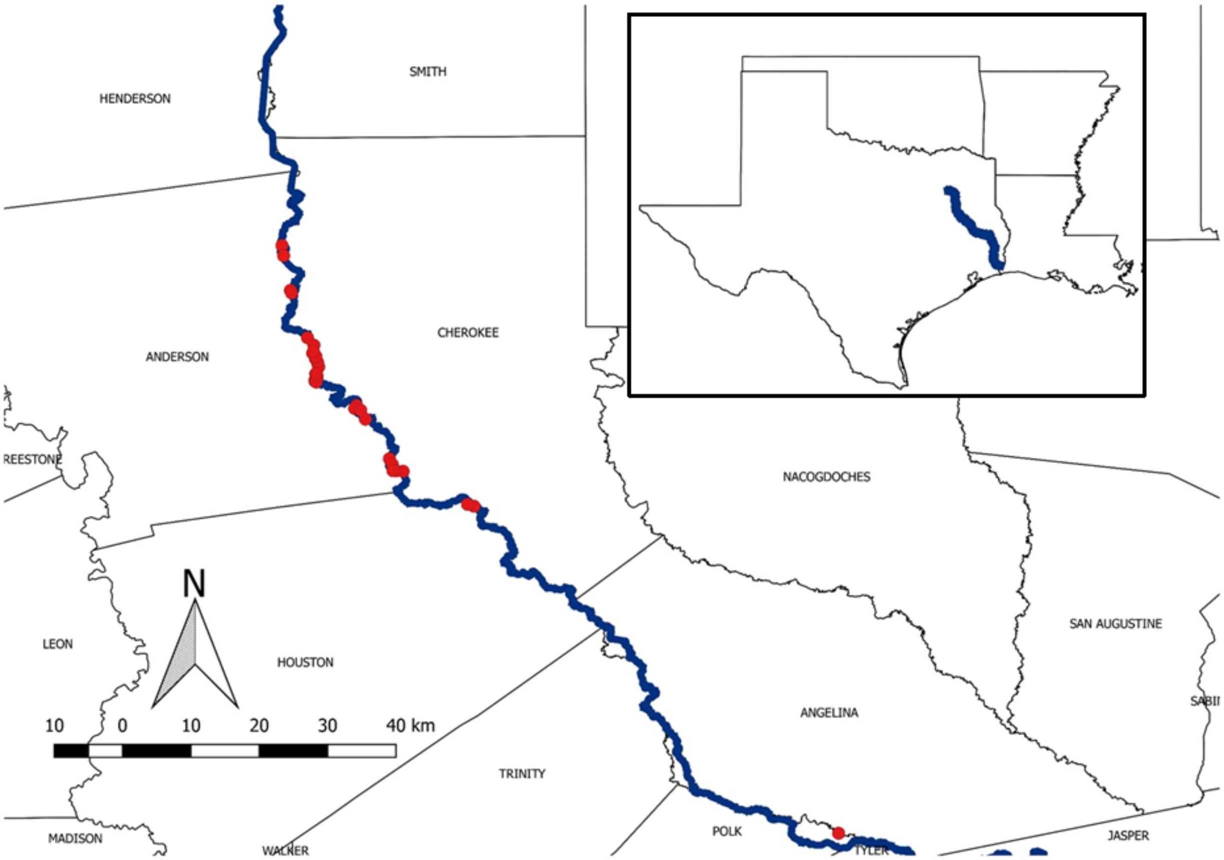

Figure 2.2.2. Illustration of canonical correspondence analysis. All environmental variables were subjected to a Pearson correlation analysis. These environmental variables explain 21.8% of the

variation in freshwater mussel distribution. Increasing entrenchment ratio corresponds to less entrenched streams.

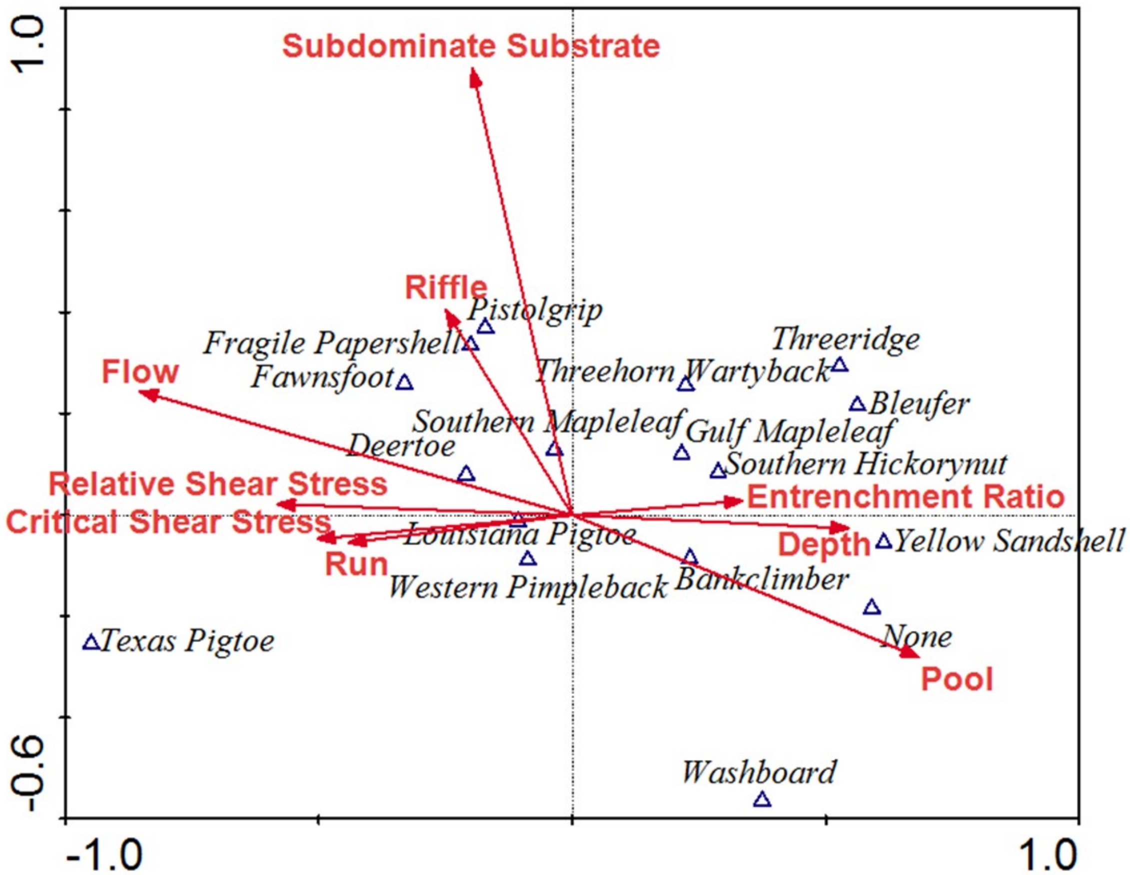

Figure 2.2.3. The range of entrenchments found along the upper Neches River. Green indicates areas of slight entrenchment and are characterized by low banks. Orange indicates areas of moderate entrenchment. Red indicates highly entrenched areas that have high banks.

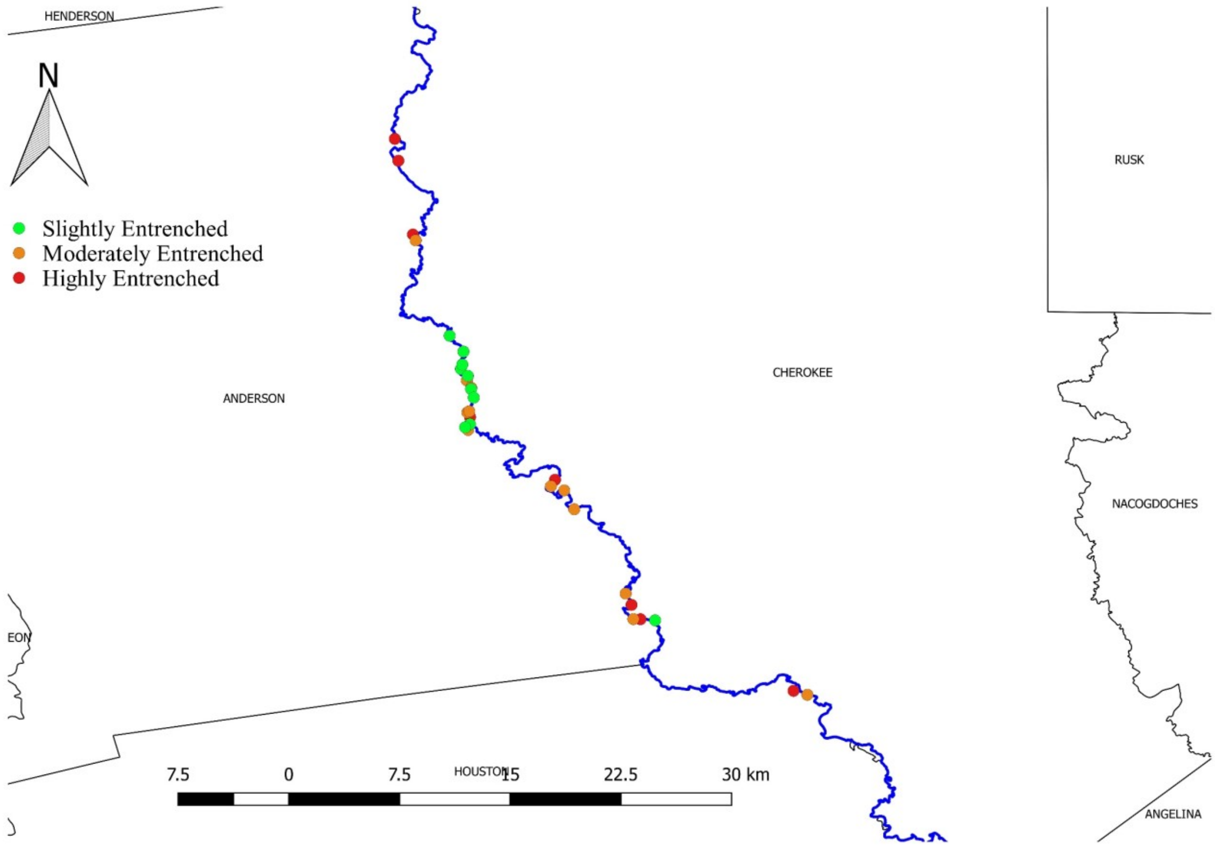

Table 2.2.1. The formulas and associated variables for each hydraulic characteristic that was calculated. Parameters were derived from Allen and Vaughn (2010). Substrate values were obtained through an in-lab sieve analysis. Depth and current velocity were obtained during field sampling using a Flo-Mate™ model 2000 and depth rod. Each hydraulic characteristic was calculated for each mesohabitat that was sampled at all 31 sample sites.

Hydraulic Characteristic	Equation	Units	Symbol	Definition
Shear Stress	$\tau=\rho(U_*^2)$	dynes/cm^2	ρ	density of water (commonly 0.998 g/cm^3)
			U_*	shear velocity (or friction velocity)
Shear Velocity	$U_*=U(5.75\ Log(12d/k_s))$	cm/s	U	mean current velocity (cm/s)
			d	water depth (cm)
			k_s	bed roughness coefficient (cm); estimated as 3.5*D_{84}
Critical Shear Stress	$\tau_c = \theta_C g D_{50}(\rho_s-\rho)$	dynes/cm^2	θ_C	shield's parameter (0.065)
			g	gravity = 980 cm/s^2
			D_{50}	Substrate particle size at which 50% is finer (cm)
			ρ_s	density of substrate (2.65 g/cm^3)
			ρ	density of water (estimated as 0.998 g/cm^3)
Relative Shear Stress	$RSS=\tau/\tau_c$	Unitless	τ	Shear Stress (dynes/cm^2)
			τ_c	Shear Velocity (dynes/cm^2)

Table 2.2.2. Mean values for each mesohabitat of all environmental variables measured in the field with calculated hydraulic variables.

	Abundance	Critical Shear Stress (dynes/m²)	Shear Stress (dynes/m²)	Shear Velocity (cm/s²)
Riffle	31.25	41.89	2.87	9.20
Run	31.93	27.73	1.94	4.66
Pool	16.57	8.74	0.58	0.53

	Relative Shear Stress	Average Flow (cm/s²)	Average Depth (cm)	Bank Angle
Riffle	0.42	37.52	57.42	21.25
Run	0.49	28.52	67.58	17.38
Pool	0.14	10.40	82.81	18.37

Table 2.2.3. Calculated values for four hydraulic variables in an east Texas stream. Shear stress (dynes/m2), critical shear stress (dynes/m2), shear velocity (cm/s2), and relative shear stress (unitless).

Mesohabitat Type	Critical Shear Stress	Shear Stress	Shear Velocity	Relative Shear Stress
Riffle	18.94	9.96	3.16	0.53
Riffle	77.87	10.37	3.22	0.13
Riffle	6.31	2.22	1.49	0.35
Riffle	77.87	4.91	2.22	0.06
Riffle	17.99	11.78	3.44	0.65
Riffle	107.34	7.97	2.83	0.07
Riffle	11.05	5.93	2.44	0.54
Riffle	12.63	3.96	1.99	0.31
Riffle	12.10	3.37	1.84	0.28
Riffle	133.65	20.83	4.57	0.16
Riffle	18.52	22.93	4.79	1.24
Riffle	8.42	6.15	2.48	0.73
Run	101.02	3.72	1.93	0.04
Run	16.84	7.73	2.78	0.46
Run	14.73	3.27	1.81	0.22
Run	17.89	5.86	2.42	0.33
Run	133.65	2.89	1.70	0.02
Run	12.63	3.40	1.85	0.27
Run	103.13	6.65	2.58	0.06
Run	11.37	1.60	1.27	0.14
Run	10.00	2.96	1.72	0.30
Run	10.00	2.66	1.63	0.27
Run	2.10	1.63	1.28	0.77
Run	21.57	3.76	1.94	0.17
Run	2.10	2.49	1.58	1.18
Run	50.51	1.77	1.33	0.04
Run	96.60	9.67	3.11	0.10
Run	2.10	2.54	1.59	1.21
Run	2.10	0.41	0.64	0.19
Run	2.42	3.06	1.75	1.26
Run	10.52	4.46	2.11	0.42
Run	8.94	24.01	4.90	2.68
Run	2.10	0.47	0.68	0.22
Run	2.10	1.59	1.26	0.75
Run	4.21	1.79	1.34	0.43

Run	6.31	3.45	1.86	0.55
Run	2.10	0.33	0.58	0.16
Run	133.65	25.28	5.03	0.19
Run	2.10	2.00	1.42	0.95
Run	19.47	4.74	2.18	0.24
Run	2.00	1.00	1.00	0.50
Pool	57.88	4.25	2.06	0.07
Pool	2.10	0.00	0.06	0.00
Pool	14.73	0.63	0.79	0.04
Pool	4.63	0.38	0.62	0.08
Pool	1.89	0.07	0.26	0.03
Pool	109.97	0.71	0.84	0.01
Pool	2.21	0.23	0.48	0.10
Pool	2.42	0.07	0.26	0.03
Pool	2.63	0.11	0.34	0.04
Pool	2.10	0.01	0.10	0.00
Pool	2.10	0.00	0.04	0.00
Pool	2.32	0.16	0.41	0.07
Pool	12.10	0.28	0.53	0.02
Pool	1.89	0.77	0.88	0.41
Pool	1.89	0.09	0.30	0.05
Pool	4.00	0.74	0.86	0.19
Pool	2.42	2.48	1.58	1.03
Pool	2.10	0.48	0.69	0.23
Pool	3.16	0.44	0.66	0.14
Pool	2.10	0.10	0.32	0.05
Pool	2.21	0.73	0.85	0.33
Pool	2.10	0.31	0.56	0.15
Pool	2.10	0.00	0.03	0.00
Pool	2.10	0.11	0.33	0.05
Pool	2.10	0.02	0.15	0.01
Pool	2.10	1.75	1.32	0.83
Pool	8.42	0.35	0.59	0.04
Pool	2.32	0.25	0.50	0.11
Pool	2.10	0.20	0.44	0.09
Pool	2.10	0.22	0.47	0.11

*Table 2.2.4. Significant outputs for the three-way log-linear contingency tables and chi-square test. P values were corrected using a Bonferroni correction, 0.05 was divided by the number of comparisons being made within each variable. * denotes a species that failed to meet the assumptions of sample size for the associated environmental variable. Bold values indicate those that are considered statistically significant.*

Species	Average Flow	Average Depth	Average Bank Angle
Bank Climber (*Plectomerus dombeyanus*)	**0.000**	0.005	0.018
Bleufer (*Potamilus purpuratus*)	**0.000**	0.053	0.035
Deertoe (*Truncilla truncata*)	**0.000**	0.013	**0.001**
Fawnsfoot (*Truncilla donaciformi*s)	**0.000**	**0.000**	0.628
Fragile Papershell (*Leptodea fragilis*)	**0.000**	**0.001**	0.027
Gulf Mapleleaf (*Quadrula nobilis*)	0.007	**0.001**	0.728
Louisiana Pigtoe (*Pleurobema riddellii*)	**0.000**	**0.000**	0.056
Pistolgrip (*Tritogonia verrucosa*)	**0.000**	**0.002**	**0.000**
Rock Pocketbook (*Arcidens confragosus*)	0.032	*	0.021
Sandbank Pocketbook (*Lampsilis satura*)	*	0.274	0.559
Southern Mapleleaf (*Quadrula apiculata*)	**0.000**	0.304	0.007
Texas Pigtoe (*Fusconaia askewii*)	**0.000**	**0.000**	*
Threehorn Wartyback (*Obliquaria reflexa*)	**0.000**	**0.001**	0.006
Threeridge (*Amblema plicata*)	**0.000**	**0.001**	**0.000**
Washboard (*Megalonaias nervosa*)	**0.000**	**0.000**	0.005
Western Pimpleback (*Quadrula mortoni*)	**0.000**	**0.000**	**0.000**
Yellow Sandshell (*Lampsilis teres*)	*	0.214	0.007

Species	Dominate Substrate	Subdominate Substrate	ER
Bank Climber (*Plectomerus dombeyanus*)	**0.003**	**0.001**	0.055
Bleufer (*Potamilus purpuratus*)	*	0.764	0.043
Deertoe (*Truncilla truncata*)	**0.003**	0.050	0.360
Fawnsfoot (*Truncilla donaciformi*s)	0.673	**0.000**	0.005
Fragile Papershell (*Leptodea fragilis*)	0.086	*	0.249
Gulf Mapleleaf (*Quadrula nobilis*)	0.066	*	0.508
Louisiana Pigtoe (*Pleurobema riddellii*)	0.815	0.009	0.358
Pistolgrip (*Tritogonia verrucosa*)	**0.000**	**0.000**	**0.002**
Rock Pocketbook (*Arcidens confragosus*)	*	*	0.739
Sandbank Pocketbook (*Lampsilis satura*)	*	*	0.572
Southern Mapleleaf (*Quadrula apiculata*)	0.154	*	0.425
Texas Pigtoe (*Fusconaia askewii*)	0.311	*	**0.001**
Threehorn Wartyback (*Obliquaria reflexa*)	**0.000**	*	0.032
Threeridge (*Amblema plicata*)	*	**0.000**	**0.001**
Washboard (*Megalonaias nervosa*)	0.015	0.011	**0.000**
Western Pimpleback (*Quadrula mortoni*)	**0.000**	**0.000**	**0.000**
Yellow Sandshell (*Lampsilis teres*)	0.406	0.146	0.202

Species	Shear Stress	Shear Velocity	Critical Shear Stress	RSS
Bank Climber (*Plectomerus dombeyanus*)	*	**0.003**	0.005	0.075
Bleufer (*Potamilus purpuratus*)	**0.008**	**0.003**	*	0.075
Deertoe (*Truncilla truncata*)	**0.000**	**0.000**	0.108	*
Fawnsfoot (*Truncilla donaciformis*)	**0.000**	**0.000**	0.128	0.802
Fragile Papershell (*Leptodea fragilis*)	**0.000**	**0.000**	0.019	0.041
Gulf Mapleleaf (*Quadrula nobilis*)	*	0.021	0.168	*
Louisiana Pigtoe (*Pleurobema riddellii*)	**0.000**	**0.000**	0.915	0.513
Pistolgrip (*Tritogonia verrucosa*)	**0.000**	**0.000**	**0.001**	0.007
Rock Pocketbook (*Arcidens confragosus*)	*	0.328	*	0.607
Sandbank Pocketbook (*Lampsilis satura*)	*	*	*	*
Southern Mapleleaf (*Quadrula apiculata*)	0.027	0.005	0.099	0.162
Texas Pigtoe (*Fusconaia askewii*)	**0.001**	**0.000**	**0.000**	**0.000**
Threehorn Wartyback (*Obliquaria reflexa*)	**0.000**	**0.000**	0.121	*
Threeridge (*Amblema plicata*)	**0.000**	**0.000**	0.007	**0.001**
Washboard (*Megalonaias nervosa*)	**0.001**	**0.000**	0.623	0.069
Western Pimpleback (*Quadrula mortoni*)	**0.000**	**0.000**	**0.000**	0.075
Yellow Sandshell (*Lampsilis teres*)	*	*	0.655	*

*Table 2.2. 5. Example of a contingency table developed to determine if habitat associations were occurring between species, mesohabitat's and environmental/hydraulic characteristics. This table investigates the relationship between mesohabitat, subdominant substrate, and Louisiana Pigtoe (*Pleurobema riddellii*). A chi-square test was conducted in order to determine if the distribution of this mussel deviates from random. P-value >0.011.*

		Subdominate Substrate				
Mesohabitat		Gravel	Pebble	Sand	Silt	Row Total
	Observed	11.00	8.00	9.00	1.00	29.00
Pool		0.32	5.87	0.83	1.74	
	Expected	0.38	0.28	0.31	0.03	0.29
		0.24	0.67	0.21	1.00	
		0.11	0.08	0.09	0.01	
	Observed	6.00	3.00	5.00	0.00	14.00
Riffle		0.01	1.04	0.13	0.14	
	Expected	0.43	0.21	0.36	0.00	0.14
		0.13	0.25	0.12	0.00	
		0.06	0.03	0.05	0.00	
	Observed	28.00	1.00	28.00	0.00	57.00
Run		0.22	4.99	0.69	0.57	
	Expected	0.49	0.02	0.49	0.00	0.57
		0.62	0.08	0.67	0.00	
		0.28	0.01	0.28	0.00	
	Column Total	45.00	12.00	42.00	1.00	100.00
		0.45	0.12	0.42	0.01	

*Table 2.2.6. contains the habitat associations derived from the three-way log linear contingency tables. * denotes no significance in the log-linear contingency tables. Average depth (m) categories (Low: 0-0.56; medium: 0.561-1.12; high: >1.121), Average flow (m/s): (Low: 0-0.21; medium: 0.2-0.44; high: >0.45), bank angle (Low: 0-11.8; medium: 11.9-23.7; high: >23.8), shear stress (dynes/cm2): (Low: 0-8.43; medium: 8.44-16.86; high: >16.87), shear velocity (cm/s): (Low: 0-1.67; medium: 1.68-3.33; high: >3.34), critical shear stress (dynes/cm2): (Low: 0-43.9; medium: 44.0-87.83; high: >87.84), relative shear stress (unitless): (Low: 0-0.89; medium: 0.90-1.79; high: >1.80), and entrenchment ratio: (Slightly: >2.20; moderately: 1.41-2.19; highly: <1.40).*

	Average Flow		Average Depth		Average Bank Angle	
Species	Mesohabitat	Category	Mesohabitat	Category	Mesohabitat	Category
Bank Climber (*Plectomerus dombeyanus*)	Run	Medium	*	*	*	*
Bleufer (*Potamilus purpuratus*)	Pool	Low	*	*	*	*
Deertoe (*Truncilla truncata*)	Run	Medium	*	*	Run	Medium
Fawnsfoot (*Truncilla donaciformi* s)	Run	Medium	Run	Medium	*	*
Fragile Papershell (*Leptodea fragilis*)	Run	Medium	Run	Medium	*	*
Gulf Mapleleaf (*Quadrula nobilis*)	*	*	Run	Medium	*	*
Louisiana Pigtoe (*Pleurobema riddellii*)	Run	Medium	Run	Medium	*	*
Pistolgrip (*Tritogonia verrucosa*)	Run	Medium	Run	Medium	Run	Medium
Southern Mapleleaf (*Quadrula apiculata*)	Run	Medium	*	*	*	*
Texas Pigtoe (*Fusconaia askewii*)	Run	Medium	Run	Low	*	*
Threehorn Wartyback (*Obliquaria reflexa*)	Run	Medium	Run	Medium	*	*
Threeridge (*Amblema plicata*)	Pool	Low	Pool	Medium	Pool	Medium
Washboard (*Megalonaias nervosa*)	Pool	Low	Pool	High	*	*
Western Pimpleback (*Quadrula mortoni*)	Run	Medium	Run	Medium	Run	Medium

	Dominate Substrate		Subdominate Substrate		Entrenchment Ratio	
Species	Mesohabitat	Category	Mesohabitat	Category	Mesohabitat	Category
Bank Climber (*Plectomerus dombeyanus*)	Run	Sand	Run	Sand	*	*
Bleufer (*Potamilus purpuratus*)	*	*	*	*	*	*
Deertoe (*Truncilla truncata*)	Run	Sand	*	*	*	*
Fawnsfoot (*Truncilla donaciformi* s)	*	*	Run	Gravel	*	*
Fragile Papershell (*Leptodea fragilis*)	*	*	*	*	*	*
Gulf Mapleleaf (*Quadrula nobilis*)	*	*	*	*	*	*
Louisiana Pigtoe (*Pleurobema riddellii*)	*	*	*	*	*	*
Pistolgrip (*Tritogonia verrucosa*)	Run	Sand	Run	Gravel	Run	Moderately
Southern Mapleleaf (*Quadrula apiculata*)	*	*	*	*	*	*
Texas Pigtoe (*Fusconaia askewii*)	*	*	*	*	Run	Highly
Threehorn Wartyback (*Obliquaria reflexa*)	Run	Sand	*	*	*	*
Threeridge (*Amblema plicata*)	*	*	Run	Gravel	Pool	Moderately
Washboard (*Megalonaias nervosa*)	*	*	*	*	Pool	Slightly
Western Pimpleback (*Quadrula mortoni*)	Run	Sand	Run	Gravel	Run	Moderately

	Shear Stress		Shear Velocity		Critical Shear Stress		Relative Shear Stress	
Species	Mesohabitat	Category	Mesohabitat	Category	Mesohabitat	Category	Mesohabitat	Category
Bank Climber (*Plectomerus dombeyanus*)	*	*	Pool	Low	*	*	*	*
Bleufer (*Potamilus purpuratus*)	Pool	Low	Pool	Low	*	*	*	*
Deertoe (*Truncilla truncata*)	Run	Low	Run	Medium	*	*	*	*
Fawnsfoot (*Truncilla donaciformi* s)	Run	Low	Run	Medium	*	*	*	*
Fragile Papershell (*Leptodea fragilis*)	Run	Low	Run	Medium	*	*	*	*
Gulf Mapleleaf (*Quadrula nobilis*)	*	*	*	*	*	*	*	*
Louisiana Pigtoe (*Pleurobema riddellii*)	Run	Low	Run	Low	*	*	*	*
Pistolgrip (*Tritogonia verrucosa*)	Run	Low	Run	Medium	Run	Low	*	*
Southern Mapleleaf (*Quadrula apiculata*)	*	*	*	*	*	*	*	*
Texas Pigtoe (*Fusconaia askewii*)	Run	Low	Run	Medium	Run	Low	Run	Medium
Threehorn Wartyback (*Obliquaria reflexa*)	Run	Low	Run	Low	*	*	*	*
Threeridge (*Amblema plicata*)	Pool	Low	Pool	Low	*	*	Pool	Low
Washboard (*Megalonaias nervosa*)	Pool	Low	Pool	Low	*	*	*	*
Western Pimpleback (*Quadrula mortoni*)	Run	Low	Run	Medium	Run	Low	*	*